11 August 2006 | $10

Science

On the Move

AAAS

Volume 313, Issue 5788

COVER

During the evolution of life, the ability to migrate and disperse became ever more sophisticated, enabling journeys thousands of kilometers long. Such travels shape a species' life history, which in turn shapes the ecology of the places visited. A special section beginning on page 775 considers some of the advances in our understanding of life on the move.

Image: Carin Cain

DEPARTMENTS

EDITORIAL

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NEWS OF THE WEEK

NEWS FOCUS

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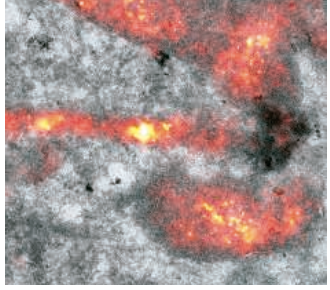

SCIENCE EXPRESS

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CLIMATE CHANGE

Satellite Gravity Measurements Confirm Accelerated Melting of Greenland Ice Sheet
J. L. Chen, C. R. Wilson, B. D. Tapley
Satellite measurements of gravity variations show that the Greenland Ice Sheet now is disappearing at the rate of about 240 cubic kilometers per year.
10.1126/science.1129007

NEUROSCIENCE

Hoxa2- and Rhombomere-Dependent Development of the Mouse Facial Somatosensory Map
F. Oury et al.
The genes that define general brain structure in the early embryo are also responsible for the organization of the neural circuit that processes sensory information.
10.1126/science.1130042

CELL BIOLOGY

Imaging Intracellular Fluorescent Proteins at Nanometer Resolution
E. Betzig et al.
Proteins of interest can be labeled with fluorescent tags and located by photoactivated localization microscopy (PALM) in thin sections and fixed cells at near-molecular resolution. >> *News story p. 748*
10.1126/science.1127344

CELL BIOLOGY

Opposing Activities Protect Against Age Onset Proteotoxicity
E. Cohen, J. Bieschke, R. M. Perciavalle, J. W. Kelly, A. Dillin
The insulin/insulin-like receptor pathway can detoxify protein aggregates in worms engineered to express excess protein in their muscles, perhaps partly explaining its role in aging.
10.1126/science.1124646

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BOOKS *ET AL.*

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Stress straddles generations.

SCIENCE**NOW**

www.sciencenow.org DAILY NEWS COVERAGE

Has the Universe Been Lying About Its Age?
Distance measurements to nearby galaxy suggest cosmos is 2 billion years older than thought.

Like Flower, Like Son
Plants remember the stresses visited upon their parents.

Just Like Mom Used to Make
Transgenic goat's milk can protect baby pigs—and perhaps someday humans—from a deadly bacterium.

Boring science?

SCIENCE **CAREERS**

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

US: No More Boring Science
GrantDoctor
NSF Director Arden Bement seems to be transforming his organization to fund only cutting-edge science.

US: Lab Dynamics—Negotiating Science
C. Cohen and S. Cohen
Whether you realize it or not, most important discussions about science are in fact negotiations.

EUROPE: Career Transition Profile—Jonathan Wood
A. Forde
Jonathan Wood's interests took him from a biology Ph.D. to editor of a materials science publication.

Puzzled by jargon and abbreviations.

SCIENCE'S **STKE**

www.stke.org SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

GLOSSARY
New terms and definitions include RHR, β-TrCP, and many more.

ST ON THE WEB
AgentCell (in Modeling Tools) is a simulation tool for exploring *E. coli* behavior and intracellular signaling.

ST ON THE WEB
Explore bacterial chemotaxis and other biochemical processes using the BCT, StochSim, or *Smoldyn* software (in Modeling Tools).

CREDITS: (TOP) B. HOHN AND J. MOLINIER; (BOTTOM) PAT N. LEWIS

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EDITED BY STELLA HURTLEY AND PHIL SZUROMI

Fishing for Change

Bottom-feeding fish in the family Prochilodontidae are the most important component of the commercial and subsistence freshwater fishery in South America, but are declining because of overharvesting, dams, and land-use changes. **Taylor *et al.*** (p. 833) show experimentally how the loss of a single Prochilodontid species will change a fundamental ecosystem-level process, the synthesis and degradation of carbon, in this species-rich tropical ecosystem. The high abundance and diversity of consumers at lower trophic levels is no "insurance" against changes in ecosystem functioning: None of the more than 100 other fish species compensated for the functional role performed by the single Prochilodontid species that was removed.

As the Rain Falls

Around 9500 years ago, the Eastern Sahara entered a much wetter phase that made it suitable for widespread human settlement. **Kuper and Kröpelin** (p. 803, published online 20 July) combine nearly 500 of their own radiocarbon dates from 150 archaeologic sites with ones previously reported to develop a detailed chronicle of habitation shifting with precipitation patterns in this region during much of the past 10,000 years. Settlements bloomed throughout the region when rainfall abruptly increased and disappeared as aridity spread from north to south until ~5000 years ago.

Puzzling X-ray Pulses

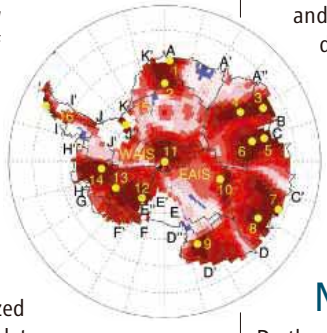
Neutron stars, the remnants of supernovae explosions, can spin on time scales of minutes or faster. However, in the center of the gas shells of the supernova remnant RCW103, which exploded just 2000 years ago, **De Luca *et al.*** (p. 814) found an unusual x-ray source pulsing with a much longer period of 6.67 hours that showed no faster variations. This object could be an x-ray binary system consisting of a compact object and low-mass star in eccentric orbit. If the object is instead a single neutron star, it could be a rare magnetar that is being slowed down, perhaps by a supernova debris disk.

When a Star Is Born

When stars condense out of gas clouds, forces other than gravity can impede their collapse. **Girart *et al.*** (p. 812; see the Perspective by **Crutcher**) show that magnetic forces can be strong enough to slow the collapse by identifying an hourglass shape in the material around a forming star. In their images taken with the Submillimeter Array, they see aligned polarization vectors pinched inward at the waist near the central star. This hourglass shape mirrors expectations from star-formation theory in which gravity eventually overcomes other forces. The polarization pattern shows that magnetism is more important in this case than turbulence in supporting the gas cloud.

Staying Even

Climate models have suggested that the amount of snow falling on the interior of Antarctica should increase as the world warms because warmer air can hold more moisture and produce more snow. Some studies that have used satellite observations or reanalyzed previous climatological data have suggested that there has been a net accumulation of snow, but a study by **Monaghan *et al.*** (p. 827) shows that no significant buildup has occurred during the past 50 years. By combining field observational data with model simulations, they provide a 5-decade-long picture of regional variability of Antarctic snowfall. Interdecadal variability of snow accumulation for the 16 regions examined was observed, but no net overall trend has resulted. Annual variability and decadal trends can be as large as, or larger than, inferred long-term trends. This finding also argues against net increases of snowfall in the interior of Antarctica mitigating global sea-level rise.



Caught in the Act

Mount Etna is one of the most active volcanoes in the world, with ongoing magma intrusions and a recent increase in explosive eruptions. In late 2002, a particularly violent outburst occurred, with fire fountains and tephra fallout. **Patanè *et al.*** (p. 821; see the Perspective by **Foulger**) caught the 2002 Etna eruption in seismic data from a dense network of receivers and were able to map changes in three-dimensional shear and pressure-wave velocity during the pre-eruptive and eruptive periods. Anomalous low-velocity zones appeared just before the eruption that were indicative of rising gas-rich magma within the volcano.

Nailing Networks

Do the networks between individuals affect how they perform as a group? **Kearns *et al.*** (p. 824) approached this question through a graph-coloring problem. Individuals had to select a color so that their choice would not overlap any of their network neighbors. Network structure had a dramatic effect on performance and, depending on the structure, providing participants with more information could decrease or increase the times individuals or groups needed to reach a solution.

Continued on page 731

CREDITS (TOP TO BOTTOM): TAYLOR *ET AL.*; MONAGHAN *ET AL.*

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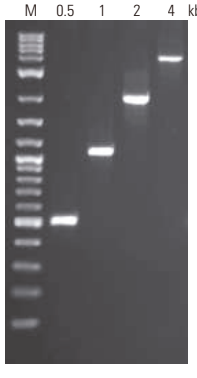
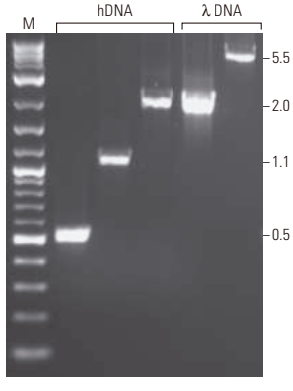


Selective and specific PCR amplification from Human Genomic DNA: Specific amplicons of 0.5, 1, 2, and 4 kb from human genomic DNA were amplified by *Taq* DNA Polymerase with Standard Buffer for 30 cycles. Marker (M) shown is 2-Log DNA Ladder (NEB #N3200).



Versatility of the *Taq* 2X Master Mix: 30 ng human genomic DNA (hDNA) or 0.1 ng lambda DNA (λ DNA) was amplified in the presence of 200 nM primers in a 25 µl volume. Marker (M) shown is 2-Log DNA Ladder (NEB #N3200).

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Continued from page 729

Tracking Smallpox

Before its eradication in 1980, smallpox was endemic around the world. **Esposito *et al.*** (p. 807, published online 27 July) sequenced 45 isolates of smallpox taken before eradication and found little variation. However, phylogenetic analysis revealed three distinct clades dividing into West African, Asian, and South American groups. These clades evolved by recombination and genome reduction, and the findings have implications for virulence. In any potential outbreak, it should be possible to trace the source.

Musseling Up Defenses

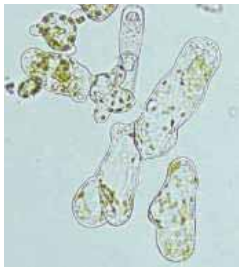
Invasive species not only alter the composition and balance of ecological communities; they can also act as selective forces. **Freeman and Byers** (p. 831; see the news story by **Stokstad**) present evidence for the rapid evolution of an inducible morphological defense in the common Atlantic mussel, *Mytilus edulis*, in response to the invasive Asian shore crab, *Hemigrapsus sanguineus*, within only 15 years of the crab's introduction. This phenomenon—a thickening of the shell when exposed to waterborne cues indicating the predator's presence—is consistent in laboratory and field experiments.

Sunrise, Sunset

To avoid navigational errors when cue availability changes because of weather conditions or time of day, the compass systems of migrating birds must be calibrated with respect to a common reference system. **Muheim *et al.*** (p. 837) provide experimental evidence in Savannah sparrows that the magnetic compass is recalibrated with respect to polarized light cues at both sunrise and sunset. In addition, recalibration of the magnetic compass occurs both before and during migration, and a view of the polarization patterns down to the horizon is required for recalibration of the magnetic compass.

Actin and Coronin in Immune Cells

The actin cytoskeleton regulates many aspects of cellular and organismal biology. Coronins have been implicated in the regulation of cytoskeletal dynamics. **Föger *et al.*** (p. 839; see the Perspective by **Dustin**) focused on understanding the in vivo functions of the actin-binding protein coronin 1. Coronin 1 was required for chemokine-mediated migration of immune cells and for organizing cytoskeletal changes.



CREDIT: ITO ET AL.

Peptide Regulators of Plant Development

Cell-cell communication is essential for organized tissue formation. Recently, a role for peptides in plant development has been established. In the shoot apical meristem of *Arabidopsis*, cell fate determination involves the *CLAVATA3* gene, which encodes a putative peptide ligand, and the *CLAVATA1* gene, which encodes a leucine-rich repeat receptor-like kinase. However, the precise identity of the individual signaling peptide has remained elusive (see the Perspective by **Simon and Stahl**). Two independent groups, **Ito *et al.*** (p. 842) and **Kondo *et al.*** (p. 845), have now isolated specific CLAVATA-derived 12–amino acid peptides involved in the regulation of plant meristem development.

Bacterial Sneak Attack

A functional nonribosomal peptide *polyketide synthase* (*pks*) gene cluster on a genomic island has been discovered in *Escherichia coli* that induces DNA double-strand breaks in the infected host-cell DNA and in turn causes a block in mitosis. **Nougayrède *et al.*** (p. 848; see the Perspective by **Hayashi**) found that the *pks* island is widely distributed in commensal *E. coli* strains and is even found in a strain used as a probiotic agent. The genotoxic effect may be exploited by the bacteria to slow the rate of renewal of the intestinal epithelium by blocking the cell cycle. Thus, the relation between pathogenicity and commensalism may be more complicated than has been assumed. These findings may provide clues about the role of microorganisms in the development of colonic cancers.

All truths are easy to understand once they are discovered; the point is to discover them.

Galileo Galilei

Italian physicist, astronomer, philosopher (1564-1642)

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Donald Kennedy is the Editor-in-Chief of *Science*

Back to the People

PRESIDENT BUSH'S RECENT VETO OF HR 810, THE MEASURE IN THE U.S. CONGRESS THAT would have expanded federal funding for stem cell research, has focused attention on what is happening in this and other issues in science policy. The Senate vote was 63 to 37 in favor: a strong vote, but neither it nor the House could gather enough votes for the supermajority required to override the veto. That left federal funds available for research on only the few cell lines derived before 9 August 2001 and revealed a seismic shift in the relationship between the president and the people's representatives in Congress. It was a surprisingly sharp rebuke to administration policy by a group including the Senate majority leader and other members of the president's own party.

National polls have repeatedly indicated that the U.S. public favors research using stem cells derived from embryos that would otherwise be discarded after in vitro fertilization procedures. That is exactly what the Senate and House legislation sought to permit, and what the president's veto forcefully rejected. Fifty-eight percent of U.S. citizens, who may know that some of our partner nations have more permissive policies, disapproved of the president's action. The interesting question we now confront is this: What happens when a clear signal from the public is unheard or unanswered by the administration in power?

Of course, standard political theory anticipates that the voters will exact their penalty at the polls. Although an opportunity of sorts will be offered by this fall's midterm elections, there is a real risk of punishing the wrong target. After all, the majority in Congress got this one right. The presidential election of 2008 looms, but it's a long distance away, and political patience is a commodity in short supply. So what might happen in the meantime?

For an explanation, we might look at some possible parallels. National polls have also shown that the U.S. public is increasingly worried about climate change and favors action at the federal level. The United Kingdom and other European nations have announced strong steps to mitigate carbon dioxide emissions, but the Bush administration has not—and it sends representatives to international meetings on the topic instructed to talk about "climate variability" rather than "climate change." There's a similarity here, and it's an unexpected one: In each case, federal failure to act has resulted in a downward migration to other jurisdictions. This may not be a unique case, but I cannot recall one like it.

In the case of climate change, states, regional cooperatives of states, and cities have begun a rebellion against the failure of national actions aimed at reducing emissions and raising fuel economy standards. The mayor of Seattle, for example, having moved his city's public transportation system to clean vehicles, has thus far gathered a consortium of 275 mayors with firm commitments to a Climate Protection Agreement with emissions reduction targets. Meanwhile, the New England states will adopt the new tailpipe standards for carbon dioxide emissions that now apply in all three West Coast states. California is even acting like a nation, as Governor Schwarzenegger forges climate-mitigation deals with UK Prime Minister Tony Blair. What's next, secession?

As to stem cells, state research initiatives were led by California's huge bond issue, passed by nearly 60% of the vote as a ballot proposition. After the Bush veto, Schwarzenegger promptly bailed out the project from a temporary legal stalemate with a $150 million state loan. Four other states have passed legislation appropriating funds for such research, and sharp struggles are under way in some others, notably Missouri, where a citizen's petition calls for a statewide referendum on the legality of embryonic stem cell research.

This outcome is an odd reversal of the federal-state tensions to which we have become accustomed. Those used to involve complaints about "unfunded federal mandates": costs that the national government lays on states by imposing obligations without paying for them. What's happening here is a turnaround: We have a "neglected federal mandate," and the states and cities are picking up the obligation cheerfully! The administration should be embarrassed by its own neglect and start listening to the voters.

– Donald Kennedy

10.1126/science.1133322

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DEVELOPMENT

When MADS, Don't Throw Tomatoes

Two types of MADS-box *APETALA3* (*AP3*) genes are found in members of the tomato family of plants: the *euAP3* group, which is critical for the proper development of the petals and stamens in angiosperms, and the less well-understood *TM6* group. *Petunia hybrida* and tomato (*Solanum lycopersicum*) each have a *euAP3* gene (*PhDEF* and *TAP3*, respectively) and a *TM6* gene (*PhTM6* and *TM6*). The *euAP3* and *TM6* lineages are hypothesized to have originated through a gene duplication event before the diversification of the major core eudicot lineages approximately 125 million years ago.

Rijpkema *et al.* and de Martino *et al.* have analyzed petunia and tomato mutants and found that in both species, *euAP3* genes maintain petal and stamen identity, whereas *TM6* genes function redundantly with *euAP3* genes in stamen development. Ectopic expression of *TM6* genes in *euAP3* lack-of-function mutants demonstrates that *TM6* genes are functionally redundant in both petal and stamen development. Rijpkema *et al.* also examined the promoter regions of *euAP3* and *TM6* regulatory sequences and found distinct yet highly conserved regions among *euAP3* core eudicot genes as well as in tomato and petunia *TM6* genes. Despite these similarities, the differences in expression and function between tomato and petunia *TM6* genes suggest that these genes have diversified functionally over a relatively short evolutionary time of 40 million years. — LMZ

Plant Cell **18**, 10.1105/tpc.106.042937; 10.1105/tpc.106.042978 (2006).

MOLECULAR BIOLOGY

Regulating the Regulators

MicroRNAs are small noncoding RNAs that regulate gene expression in eukaryotes by targeting homologous sequences in messenger RNAs, but less is known about how the synthesis of miRNAs is regulated. To begin with, miRNA genes are transcribed by RNA polymerase II. After transcription, miRNAs undergo a complex maturation process: (i) the primary miRNA, or pri-miRNA, is cleaved by the nuclear enzyme Drosha into a stem-loop precursor called a pre-miRNA, and (ii) the pre-miRNA is exported to the cytoplasm and cleaved by Dicer into the mature 22-nucleotide miRNA.

Mouse let-7 miRNAs are strongly induced during embryonic development, and the levels of pre-miRNA and mature miRNA change coordinately. In contrast, Thompson *et al.* show that for several of these same let-7 miRNAs, the levels of pri-miRNAs are constant during embryogenesis, suggesting that pri-miRNA maturation is being regulated at the Drosha processing step, and that this is also true for a number of other developmentally regulated mouse miRNAs. Intriguingly, the generalized down-regulation of miRNAs in cancer may be due to a block at the Drosha processing step. Together with previous evidence that miRNA levels can be controlled at the stage of Dicer cleavage, regulating pri/pre-miRNA processing provides a further mechanism for tightly constraining the expression of developmentally potent (and thus potentially dangerous) miRNAs. — GR

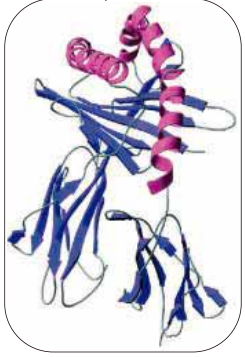
Genes Dev. **20**, 2202 (2006).

VIROLOGY

Now You See It, Now You Don't

When a cell is infected with a virus, it can alert the host immune system by expressing telltale markers on its surface. Natural killer T cells recognize these markers and kill the infected cell, preventing viral replication and stopping infection. Yuan *et al.* studied cells infected with herpes simplex virus 1 (HSV-1) and found that the virus reduced the surface expression of CD1d molecules, the proteins that bind viral lipids and present them to natural killer T cells during antiviral defense. It did this not by reducing synthesis levels nor by promoting endocytosis from the cell surface, but instead by preventing the recycling of internalized CD1d to the cell surface and diverting CD1d to the lysosomal membrane. Reducing the levels of CD1d at the cell surface reduces the ability of the infected cells to stimulate natural killer cells and helps HSV-1 to evade the immune surveillance machinery, particularly during latent infections. — SMH

Nat. Immunol. **7**, 835 (2006).



Structure of CD1d.

CHEMISTRY

Cope in a Capsule

One goal of self-assembly research is to prepare synthetic structures of sufficient complexity to achieve the remarkable catalytic rate accelerations and selectivities characteristic of enzymes. Fiedler *et al.* explore the capacity of self-assembled tetrahedral capsules to catalyze a unimolecular reaction—the 3-aza Cope rearrangement of allyl enammonium cations. Each capsule is composed of four gallium centers bridged by catecholamide ligands and bears a 12-negative charge that attracts the cationic reagent to the interior but reduces affinity for the neutral hydrolyzed product.

The authors previously found that the capsules induced ~100-fold to ~1000-fold rate increases relative to the uncatalyzed reaction; temperature-dependent kinetic studies of an ethyl-bearing substrate suggested that the acceleration was due purely to decreased entropy of activation. Extending the kinetic studies to additional substrates reveals that although entropy factors continue to play a major role, in some cases the capsules reduce activation enthalpy as well. Analysis of nuclear

Continued on page 737

CREDITS (TOP TO BOTTOM): RIJPKEMA *ET AL.*, *PLANT CELL* **18**, 10.1105/TPC.106.042937 (2006); ZENG *ET AL.*, *SCIENCE* **277**, 339 (1997)

Continued from page 735

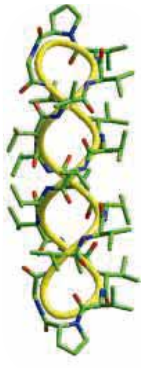
Overhauser effects in nuclear magnetic resonance spectra supports a mechanism in which the capsule binds substrates in particularly reactive conformations. Additional kinetic studies at variable hydroxide concentration suggest that the hydrolysis step takes place outside the capsule, through the intermediacy of a tight ion pair. Because the capsules are chiral, the authors suggest that further refinement may allow efficient diastereoselection or enantioselection in reactions of substrates that lack binding sites for more traditional molecular catalysts. — JSY

J. Am. Chem. Soc. **128**, 10.1021/ja062329b (2006).

CHEMISTRY

Pinning Down β Helices

The β-helical motif, which is formed by alternating D and L amino acids, has been pursued less often in small-peptide design than the more familiar α and 3_{10} helices, in part because the peptide can remain single-stranded or produce a mixture of parallel and antiparallel forms. Sastry *et al*. have designed and chemically synthesized β hairpin/β helix cyclic peptides with 5.6 residues per turn that form antiparallel helices in organic solvent. In the two peptides, two strands of either Val or Leu residues of alternating handedness are joined by two Pro-Gly hairpins and stabilized by 16 hydrogen bonds; circular dichroism spectroscopy confirmed that the sequences chosen create a left-handed Leu helix and a right-handed Val helix. Analysis of nuclear magnetic resonance spectra and amide vibrations in the infrared absorption spectrum indicated that the antiparallel helices are quite stable in solution, but that a variant with only one β hairpin exists in multiple conformations. The authors suggest that derivatives with more hydrophilic amino acids should exhibit similar stability in aqueous media. — PDS



β hairpin/ β helix motif.

CREDIT: SASTRY *ET AL.*, *J. AM. CHEM. SOC.* **128**, 10.1021/JA062737F (2006)

J. Am. Chem. Soc. **128**, 10.1021/ja062737f (2006).

BIOPHYSICS

Spacing Out the Doughnuts

Recent innovations in fluorescence microscopy have brought within reach the goal of being able to image the internal workings of live cells at a resolution of 10 nm (see, for example, Betzig *et al.*, *Science* Express, Reports, 10 August 2006). Donnert *et al.* report the latest improvement in their approach, called stimulated emission depletion (STED) microscopy, which relies on an annular pulse that de-excites fluorophores around a central spot. In order to de-excite molecules in the doughnut-shaped area thoroughly and rapidly, relatively high intensities were needed, which increased the danger of photobleaching. They have now developed a paired-pulse delivery schedule (0.25 MHz) of the excitation (100 ps) and de-excitation (280 ps) beams, where the pulse duration is long enough to return excited molecules in higher singlet states to S_0 and the pulse frequency is low enough so that triplet states relax before the next pulse arrives. The reduction in data acquisition time is largely compensated for by a higher intensity de-excitation beam and an increase in fluorescence yield, with roughly one-sixth of all fluorophores in the spot being excited to S_1. — GJC

Proc. Natl. Acad. Sci. U.S.A. **103**, 11440 (2006).

COMPUTER SCIENCE

iTunes Meets Wikipedia

The organizing efficiency offered by searchable electronic databases has long been among the most useful features of modern computers. Compared with organizing text files, however, assembling a searchable multimedia database of recorded music is a daunting task. A musicologist would like to be able to type in "Mozart" and "piano sonata" and get as output a list of recordings sorted by performing artist and a selection of stored musical scores. The researcher might then like to synchronize each recording with the score so that when replayed, the recording would follow the score precisely as shown on the screen. Ideally, playing a few notes on an interfaced musical keyboard would cause the system to zero in on a particular passage.

Dunn *et al.* explain that such a fully functional system may be a decade away from realization. Nonetheless, their work on a system called Variations2 is gradually leading to more powerful music storage and retrieval environments, in which nontextual objects such as sound recordings are linked with graphical objects such as musical scores (which may exist in numerous editions) and the underlying sequences of musical notes. The researchers say that the next version, Variations3, will improve content-based searching of musical works and add better support for non-Western music. Such research could also provide valuable general strategies for navigating a wide range of nontextual data. — DV

Commun. ACM **49**, 53 (2006).

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Information for Contributors

See pages 102 and 103 of the 6 January 2006 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

DATABASE

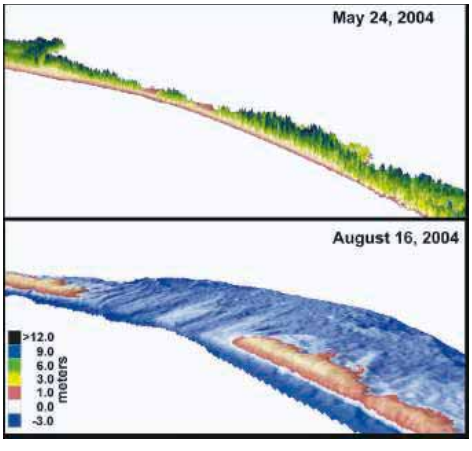
Carcinogen Hunt

Benzene and DDT make the list of compounds that cause cancer in lab animals, but caffeine doesn't. For an exhaustive roundup of this research, check out the Carcinogenic Potency Database from Lois Swirsky Gold, Bruce Ames, and colleagues at the University of California, Berkeley. The site collates data on the cancer-causing ability of 1485 compounds, drawing on more than 6000 animal tests from the 1950s through the 1990s. A chart atop each compound's page summarizes the results and, if they are positive, lists which organs develop tumors and the dose that spurred cancer in half of the animals studied. Read further for a synopsis of experiments on the substance. The authors are known for arguing that the risk to humans from synthetic chemicals is overstated, but their views don't color the site's coverage. >>
potency.berkeley.edu/cpdb.html

DATABASE

Wild Europe

Supervised by four natural history museums, Fauna Europaea is a taxonomic storehouse covering all of the continent's terrestrial and freshwater animals. It offers classification information and range maps for species such as the genet (*Genetta genetta*), a slinky cousin of the mongoose, and the parasitic flatworm *Diplozoon paradoxum*, which sups on the gills of fishes. >> www.faunaeur.org



CREDITS (TOP TO BOTTOM): NOAA OCEAN EXPLORER; USGS; MICHAEL THORPE/ASU

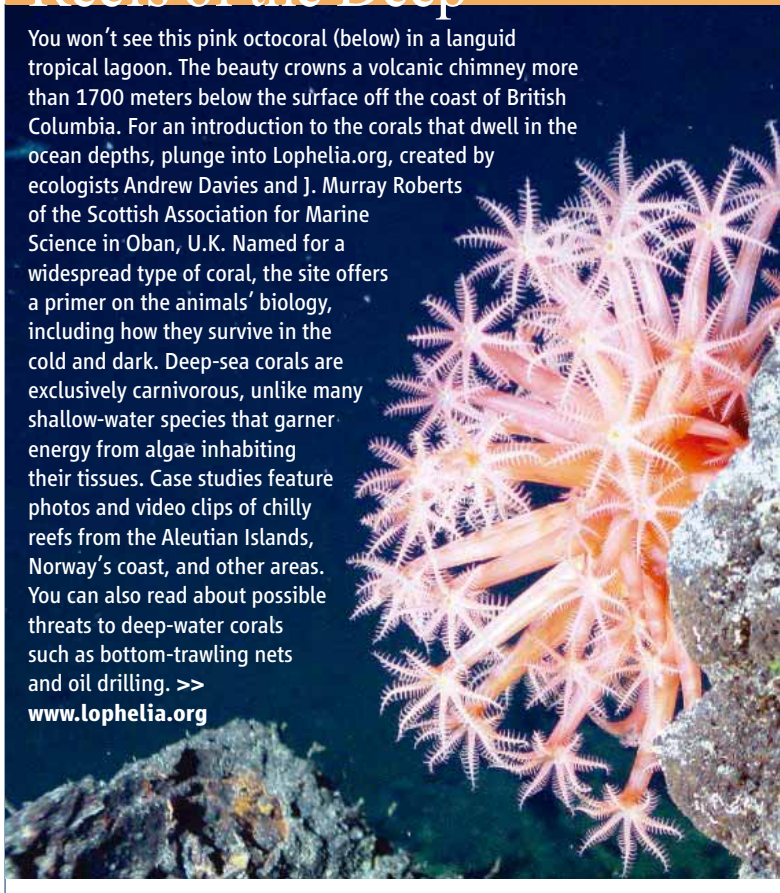
EDUCATION

<< Earth, Wind, And Fire

The new Natural Hazards Gateway from the U.S. Geological Survey (USGS) offers students and the general public quick access to data on an almost biblical array of disasters, including hurricanes, earthquakes, wildfires, and floods. Each of the seven sections supplies fact sheets on a specific hazard and posts the latest alerts and activity reports. You'll also find plenty of links to other, mainly USGS, sites at which you can nab background information and up-to-date conditions. Wade into the flood section, for instance, and you'll be deluged by real-time stream-flow values from around the country. Another highlight is the hurricane impact studies, which feature dramatic images from recent storms. These before-and-after maps (above) based on laser altimetry, or lidar, show that Hurricane Charley severed Florida's North Captiva Island in 2004. >>
www.usgs.gov/hazards

RESOURCES

Reefs of the Deep

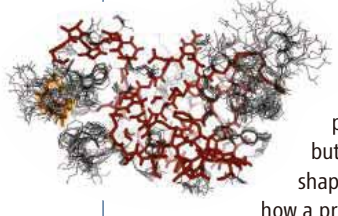


You won't see this pink octocoral (below) in a languid tropical lagoon. The beauty crowns a volcanic chimney more than 1700 meters below the surface off the coast of British Columbia. For an introduction to the corals that dwell in the ocean depths, plunge into Lophelia.org, created by ecologists Andrew Davies and J. Murray Roberts of the Scottish Association for Marine Science in Oban, U.K. Named for a widespread type of coral, the site offers a primer on the animals' biology, including how they survive in the cold and dark. Deep-sea corals are exclusively carnivorous, unlike many shallow-water species that garner energy from algae inhabiting their tissues. Case studies feature photos and video clips of chilly reefs from the Aleutian Islands, Norway's coast, and other areas. You can also read about possible threats to deep-water corals such as bottom-trawling nets and oil drilling. **>> www.lophelia.org**

SOFTWARE

Flex Time

Like a beginning yoga student, some molecules just can't bend into certain positions. Chemical bonds and hydrophobic interactions can lock up a section of a protein or other macromolecule and prevent it from flexing and rotating. Researchers can home in on limber and stiff molecular segments with the program FIRST from biophysicist Michael Thorpe's group at Arizona State University, Tempe. Free for academics, the software doesn't predict how a protein or DNA strand will fold, but it can quickly determine the range of possible shapes. Such information is useful to scientists studying how a protein binds to a drug, or how the shell of a virus takes shape. In this image of the bacterial enzyme barnase (above), red denotes the rigid strands. >> flexweb.asu.edu



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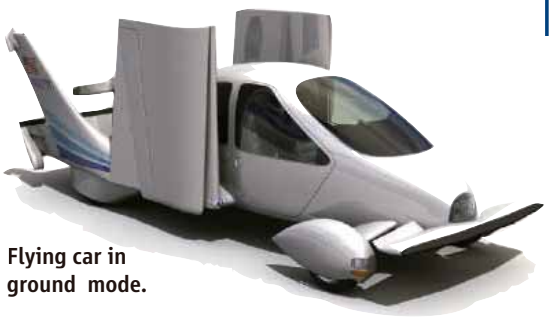
READY FOR TAKEOFF?



Flying car in ground mode.

In 1940, Henry Ford confidently predicted that "a combination airplane and motorcar" was just around the corner. Sixty-six years later, a company founded by three Massachusetts Institute of Technology (MIT) grads claims to be close to delivering on the automaker's promise.

Last month, the Cambridge, Massachusetts–based company Terrafugia took its wind-tunnel-tested and computer-simulated air-car plans to the annual Airventure air show in Oshkosh, Wisconsin. Called the Terrafugia Transition, the two-passenger vehicle, which would be the size of a large sport/utility vehicle, now exists in one-fifth-size scale models.

The company promises that a Transition driver/pilot could drive to the airport, unfold the 8-meter wings at the push of a button, and take off without lifting the wheels. Driven by a propeller in the rear, the vehicle is supposed to be able to fly up to 800 kilometers on a tank of automobile gas, going at 190 km per hour at a cruising altitude of up to 4200 meters.

Company founder Carl Dietrich and colleagues say they rethought the problem as one of "making a plane that can drive" instead of the usual "car that can fly" approach. MIT aeronautical engineer John Keesee (who has no ties to Terrafugia) says that the company hasn't come up with anything revolutionary, but "they've put together a lot of maturing technologies," such as a fiberglass and composite fuselage, "that have the capability to make it all work."

OMINOUS BEAUTY

These nacreous clouds, photographed over Australia's Mawson Station in Antarctica on 25 July, are a lovely sight, but they bode ill for the ozone layer. So named because they resemble the inside of a mother-of-pearl shell, nacreous clouds have taken on new significance over the past few decades as levels of the pollutant chlorine have increased. These ice clouds form in the –90°C cold of the Antarctic winter. They contribute to the ozone hole by triggering chemical reactions that process chlorine into a form that can destroy ozone once the first sunlight strikes in the spring.

This year, says ozone researcher Paul Newman of NASA's Goddard Space Flight Center in Greenbelt, Maryland, temperatures are unusually low out toward the expected hole's periphery. Because the destructive reactions are ultimately cold-dependent, that may portend a larger-than-average hole this year, he says.

Ruler Laid Low by Gout

The mummified fingertip of Charles V of Spain testifies to the half a lifetime of pain endured by one of the most powerful rulers of the Middle Ages.

Charles V, Holy Roman emperor from 1519 to 1556, reputedly suffered from painful gout starting at the age of 28. This limited his ability to travel and to write and caused him to give up the throne at the age of 56. He died at 58 and was buried near El Escorial monastery in San Lorenzo. Before being entombed, one of his pinky fingertips was cut off and preserved as a religious relic.

Radiograph of fingertip.

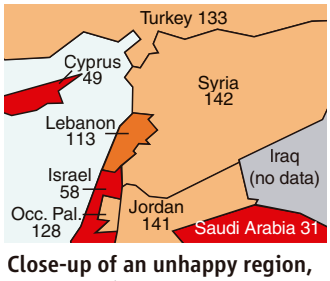
To verify what ailed the emperor, a team led by Pedro Fernández, a pathologist at the University of Barcelona, persuaded church officials to turn over a piece of the relic. Using an electron microscope, they found that the flesh was infiltrated by needle-shaped crystals of uric acid, typical of gout. By the end of his life, Charles's finger joints were probably destroyed by crystal-packed growths known as gouty tophi, the team reported in the 3 August *New England Journal of Medicine*.

"The evidence is totally convincing," says Philip Mackowiak, a pathologist at the University of Maryland School of Medicine in Baltimore. The emperor, he notes, was very fond of meat and drink, which exacerbate the condition, but it could also stem from the lead used at the time to preserve wine and to line water pipes.

SCIENTIST COMPLETES HAPPYMAP

The first world map of happiness is here. By analyzing data from more than 100 different studies, psychologist Adrian White of the University of Leicester, U.K., has created a picture of global well-being. Denmark was number one, followed by Switzerland and Austria. African and former Soviet bloc countries emerged as the most miserable.

Surveys about people's satisfaction with life were analyzed in conjunction with data on health, wealth, and access to education. Health correlated best with well-being, says White, who hopes his project will be helpful as governments have shown increasing interest in the concept of happiness.

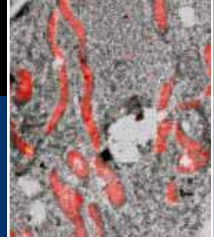


Close-up of an unhappy region, with White's rankings.

Economist Paul Dolan of Imperial College London cautions that it is difficult to compare happiness between countries. People in Asia, for example, consistently report less happiness than do those in South America—possibly because of differing cultural values placed on happiness. For a clickable world map, go to www.le.ac.uk/pc/aw57/world/sample.html.

CREDITS (TOP TO BOTTOM): TERRAFUGIA; RENAE BAKER 2006; *NEW ENGLAND JOURNAL OF MEDICINE* (2006); ADRIAN WHITE/UNIVERSITY OF LEICESTER

Viral stowaway
747



Beating the diffraction barrier
748

GEOPHYSICS

Stealth Tsunami Surprises Indonesian Coastal Residents

The earthquake that drove a tsunami onto the Indonesian island of Java last month, killing more than 600 people, packed a deceptively weak seismic punch, but it spawned a surprisingly big tsunami. That rare and poorly understood combination proved treacherous for those on the Java coast. Most took little notice of the feeble shaking, and few, far too few, made the connection to an impending killer wave. The deceptively mild quake only accentuated the lesson of the great Sumatran tsunami of 2004: Those living by subsea earthquake country should learn to interpret even the subtlest cues from the land and sea.

The 17 July quake 200 kilometers offshore of the city of Pangandaran was one in 100, a so-called tsunami earthquake capable of pushing a far bigger wave onshore than normal quakes of the same magnitude. Seismologists suspected as much when they revised their initial magnitude estimate based on high-frequency, ground-shaking seismic waves upward to magnitude 7.7. The revision added in the energy of low-frequency waves that can sway distant skyscrapers but go little noticed on the ground. Most of the quake's energy was released in low-frequency waves. And seismologist Chen Ji of the University of California, Santa Barbara, calculated that the fault beneath the deep-sea Sunda Trench ruptured at a speed of 1.1 kilometers per second, one-third the velocity of normal earthquakes, another hallmark of a tsunami earthquake.

The slow rupture velocity suggests to seismologists that the quake was cutting through something weak that would bog down any rupture, likely one-time bottom sediments not yet entirely squeezed into brittle rock. The less energy that goes into breaking the rock, the more that goes into ▶

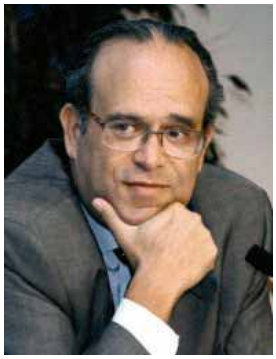
Practice run. During a drill, Central Java residents evacuate up steps built since the latest tsunami.

FRANCE

Cancer Chief Calls It Quits After Controversy

PARIS—After less than 15 tumultuous months on the job, the controversial first head of France's National Cancer Institute (INCa) has resigned. David Khayat, 49, announced last week that he is going back to his post as head of oncology at the Pitié-Salpêtrière Hospital in Paris. His departure leaves unresolved fundamental questions about the role of the institute, a recent political invention, in France's research landscape.

Khayat became the center of a French media storm earlier this year, when a widely publicized anonymous letter claimed lavish spending patterns, nepotism, and other malversations at INCa. An independent investigation by auditors from the finance ministry, whose results were released in June, cleared Khayat of any wrongdoing. But the auditors did criticize his management, as well as INCa's organizational structure and its ill-defined role.



Beleaguered. David Khayat has stepped down from INCa.

INCa was one of the key components of a grand "Cancer Plan," unveiled in 2003 by President Jacques Chirac, to whom Khayat is close. Under its unusually broad mandate, INCa coordinates France's entire war on cancer, including educating patients and politicians, implementing prevention strategies, improving patient care, and research. Its annual budget is $125 million.

Daniel Louvard, director of the Curie Institute in Paris, says Khayat failed to communicate effectively and tried to bring all of France's cancer research under his control, which led to a series of conflicts. For instance, Khayat wanted INCa to share in the intellectual property rights of the research if funded, which led many research organizations to temporarily refuse INCa grants. (The audit advised against Khayat's position, and he has backed down.) Khayat seemed to take little interest in the opinions of INCa's international scientific council, adds Louvard, a member of that group.

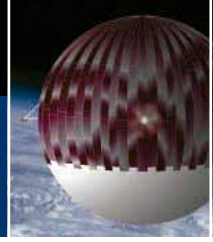
But Dominique Maraninchi of the Paoli-Calmettes Institute in Marseille, who chairs the scientific council, disagrees. On the whole, he says, Khayat "did a fantastic job setting up the organization." Khayat has also taken important steps to improve care for patients, says Henri Pujol, president of the League Against Cancer, who also sits on INCa's 27-member board of directors.

An INCa spokesperson said that Khayat was on vacation last week and would not talk to the press. In interviews before his resignation, however, Khayat has defended his management as "impeccable"; an INCa press release also points out that Khayat had announced when he was appointed that he would not stay long. The person most often mentioned as a possible successor is Maraninchi, who says he's "ready to do the job." **–MARTIN ENSERINK**

CREDITS (TOP TO BOTTOM): COSTAS SYNOLAKIS; OECD PHOTO OCDE

Stringing physicists along 750

New life for old museum 754



Who needs space? 756

sliding one side of the fault past the other. And the farther the sea floor slides upward in a trench quake—lifting the water above it into a wave—the bigger the tsunami. Add in the trench's deep water—making for more water to squeeze up into a wave on reaching shore than if the quake struck shallow waters near land—and the Java coast was in for a relative whopper of a tsunami. The biggest wave flooded the coast with several meters of water.

While nature was working against the Javanese by generating an outsized tsunami, it was also busy concealing what it had in store. The trench lies relatively far offshore, maximizing the damping effects of distance on shaking at the coast. In addition, because the quake spent most of its energy in low-frequency waves, it lacked the crackle and pop of quakes that break strong, brittle rock. To survivors, the seemingly feeble quake—if it was felt at all—didn't recall the catastrophes that followed offshore quakes to the north in December 2004 and March 2005, according to scientists and journalists. Even the precursory receding of ocean waters came at low tide, masking the approach of the first inundating wave crest.

Although the unusual nature of the quake was working against coastal residents, they didn't get much help from their government before the tsunami struck. "The message from the 2004 disaster has been largely lost," says tsunami researcher Costas Synolakis of the University of Southern California in Los Angeles. "There has been little or no education and little or no planning" on the coast, he adds. So locals were on their own to interpret the unusually subtle signs of an impending tsunami. The Pacific Tsunami Warning Center in Hawaii did issue a watch 17 minutes after the quake, noting the possibility of a local Java tsunami, but word did not reach the coast before the first wave hit 5 or 10 minutes later. Even when a high-tech warning system arrives in a few years, say researchers, the best bet may still be to educate the public in the sometimes subtle ways of earthquakes and their tsunamis.

–RICHARD A. KERR

SCIENCE EDUCATION

Evolution Trumps Intelligent Design in Kansas Vote

Defenders of evolution are set to regain control of the Kansas Board of Education and overturn the state's science standards, which are widely seen as favoring the teaching of intelligent design (ID). But they are uncertain whether their return to power, 2 years after being outvoted by ID proponents, will end the political Ping-Pong the two sides have been playing since 1999.

In Republican and Democratic primaries conducted last week, pro-evolution candidates won party nominations for three of the five board seats that are up for reelection in November. Three of the board's other five seats are held by moderates. The results mean that, regardless of the individual winners in the November election, the board's composition will flip from its existing 6–4 conservative tilt to at least a 6–4 majority controlled by moderates.

"This is a great day for Kansas," Sally Cauble, a moderate who won the Republican primary in western Kansas, told *Science* the day after the election. The former elementary school teacher from Liberal, Kansas, had a tough race against incumbent Connie Morris, who has mocked evolution as "a nice bedtime story." After a busy campaign during which she drove some 48,000 km up and down her district, Cauble won 54% to 46%.

If she wins in November, Cauble wants to vote out the pro-ID standards that were adopted last year in favor of standards issued earlier by a panel of scientists and teachers appointed by the board. Those standards, rejected by the current board, emphasize the teaching of evolution.

Middle ground. Sally Cauble (at podium), a moderate Republican running for the Kansas state education board, says schools should teach evolution but allow students to question it.

CREDIT: CHARLIE RIEDEL/AP PHOTO

But Cauble does not see a lasting solution, which is why she advocates a softer stance in combating ID. "Parents are okay with teaching evolution in public schools as long as we don't stop children from questioning it. If children ask about creationism, we need to tell them that that's a question they should ask their families and their church," she says. "We need to let the public know that science tests evolution every day, and evolution keeps proving itself."

Nobody expects the controversy to die when the new board takes over. Kansas has seen a seesaw battle over the issue since 1999, when conservatives introduced creationism into the standards. Those standards were thrown out when moderates took control of the board in 2002. Two years later, the conservatives made a comeback.

John Bacon, one of the two pro-ID incumbents who won last week's primaries, promises that the issue won't go away. "It's unfortunate that we'll now be forced to again teach evolution as the only possible explanation for the origin of life," he says.

Jack Krebs of Kansas Citizens for Science says ending the controversy will require a broader social dialogue "about the relationship between God and nature." The ID movement, he says, has driven Kansans to think that they need to choose between religion and science: "Mainstream theists and others need to speak up for the compatibility between the two."

–YUDHIJIT BHATTARCHARJEE

IMAGING

Brilliant X-rays Reveal Fruits of a Brilliant Mind

Passages written by the ancient Greek mathematician Archimedes, hidden for nearly 800 years, returned to view over the past 2 weeks, thanks to researchers at the Stanford Synchrotron Radiation Laboratory (SSRL) in Menlo Park, California. The scientists used the synchrotron's hair-thin beam of x-rays to light up the Archimedes text, which was originally copied by a 10th century scribe onto goatskin parchment. Three centuries later, a monk scraped off the Archimedes text, turned the pages sideways, and copied Greek Orthodox prayers onto the recycled pages. Although Stanford's analysis of the text hasn't yet revealed any obvious revolutionary surprises, researchers did find a new geometric drawing as well as several previously missing passages.

Rare find. This Medieval prayer book conceals seven treatises by Archimedes, two of them unique.

"Nothing usually jumps out with Archimedes," says William Noel, the curator of manuscripts and rare books at the Walters Art Museum in Baltimore, Maryland, who is leading the restoration effort. "It takes blood, sweat, toil, and tears to get at what is there." Nevertheless, he adds, "people will be talking about what we are discovering now in 100 years' time and still arguing about it."

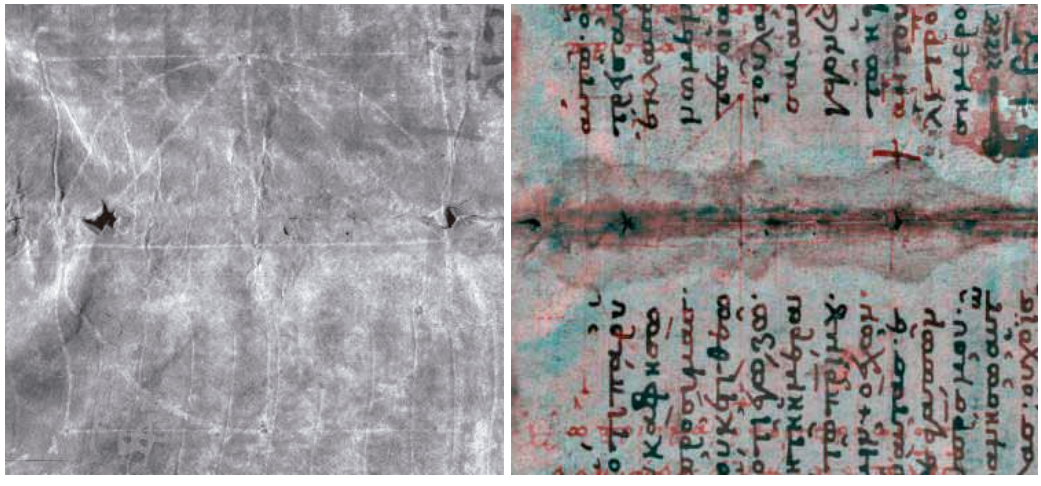
Few dispute that Archimedes was one of the world's greatest mathematicians. Today, he's known primarily for the legendary exclamation of "Eureka!" when he realized he could measure the volume of objects by figuring out how much water they displace. But he also helped create a rudimentary form of calculus 20 centuries before Newton and Leibniz put quill to paper. He came up with a way to calculate the value of pi and was the first to tackle the concept of infinity. And Archimedes's understanding of physics helped him invent the catapult and other defenses that his city-state of Syracuse used to repel Roman invaders until 212 B.C.E., when the city was finally overcome and Archimedes was killed.

The 174-page hidden manuscript, known as the Archimedes palimpsest, was discovered in 1906 by Danish classics professor John Heiberg, who used a magnifying glass to painstakingly decode the nearly invisible underlying text. But much remained undeciphered, and the book soon disappeared into a private collection. The manuscript resurfaced in October 1998 when it was sold at auction to an anonymous buyer for $2 million. By then it had been severely damaged by mold. Forged gold leaf paintings, completely covering four pages, had also been added, probably in hopes of increasing the prayer book's value.

The day after the book's sale, Noel read about the auction in a *New York Times* article that mentioned the book's dealer. Noel e-mailed the dealer, who eventually put him in contact with the owner, who later agreed to lend the book to the Walters Art Museum for restoration and imaging. Noel says that the owner has paid for the entire project, although the amount spent has not been made public.

Noel and his colleagues from Johns Hopkins University in Baltimore, Maryland, and the Rochester Institute of Technology in New York originally used multispectral imaging to reveal much of the underlying Archimedes text. Although largely successful, the visible and ultraviolet light were unable to peer beneath the forged paintings or to resolve other passages in the faint text. In 2003, Uwe Bergmann, a physicist at SSRL, came up with the idea of scanning synchrotron x-rays over the document to reveal elements such as iron and calcium in the residual ink. The energy of the x-rays is tuned to kick out inner electrons from those elements, Bergmann explains. That disruption triggers outer electrons to drop into the vacancies, giving up their excess energy as x-rays with a characteristic energy for each element, which are then captured by a detector. Computer programs then convert the steady stream of detected x-rays into gray-scale or color-enhanced images to reveal the hidden text.

The current round of imaging was successful, Noel says, and revealed numerous previously hidden passages, which can be viewed at www.archimedespalimpsest.org. In one section on mathematical propositions in a treatise titled *Method of Mechanical Theorems*, for example, Archimedes used infinite numbers to help him calculate volumes of particular objects. Although much of that text had been revealed by multispectral imaging, "there have been gaps in our reading," says Reviel Netz, a historian of ancient science at Stanford University in Palo Alto, California. "It seems the new [x-ray] images will definitely contribute to settling the reading."



Eureka. Synchrotron x-rays tuned to reveal calcium brought to life text and drawings (*left*) that multispectral imaging had shown to be lurking beneath later writings by Byzantine scribes (*right*).

The new x-ray technique "is absolutely fabulous" for recovering palimpsest texts, says Nigel Wilson, a classics scholar at Oxford University in the U.K. It's particularly exciting, he says, because many palimpsests remain to be studied. **–ROBERT F. SERVICE**

EVOLUTION

Native Mussel Quickly Evolves Fear of Invasive Crab

When an invasive species arrives, many ecologists fear the worst: a new creature running amok through an ecosystem and driving native species extinct. "People have the idea that it's a bloodbath," says Geoffrey Trussell, an evolutionary ecologist at Northeastern University in Boston, Massachusetts. "The assumption has been that prey just passively submit to their fate on the dinner plate."

Some species refuse to roll over, however, and even improve their defenses. On page 831, Aaren Freeman, a Ph.D. student in zoology at the University of New Hampshire, Durham, and his adviser James Byers describe how a native mussel of New England has rapidly evolved the ability to shield itself from an invasive crab. "It doesn't mean that we ought to ignore the threats of these introductions, but it does show that native species are not helpless," says George Cox, a retired biologist in Santa Fe, New Mexico, and author of *Alien Species and Evolution*.

Tough nut. Mussels that grow a thicker shell have a better chance of surviving an attack by the invasive Asian shore crab.

The invader in this case is the Asian shore crab (*Hemigrapsus sanguineus*), which turned up on the New Jersey coast in 1988. Since then, it has bred prolifically and spread to North Carolina and midway up the coast of Maine. The 4-centimeter-wide marine crab, which has a broad diet, has acquired a taste for the blue mussel (*Mytilus edulis*), which people eat as well. These mussels already have to deal with another invader, the green crab (*Carcinus maenas*), which arrived from Europe in the 1800s and has established itself along the East Coast.

Mussels, of course, can't flee predators. So when young blue mussels sense that the green crabs are near their particular patch—no one knows the telltale signal, but it's likely a hormone or other chemical—they begin to thicken their shells. After several months, the shell is 5% to 10% thicker than it would otherwise have been. This seems to help, as crabs need 50% more time to open mussels with thicker shells. "Crabs often will give up if they can't open a mussel and move on to easier prey," Freeman says. If crabs don't happen to be around, the mussels don't bother making thicker shells, perhaps because it diverts energy from other activities, such as reproducing.

Freeman and Byers wanted to know whether the mussels were also able to detect the recently arrived Asian shore crab. For their experiment, they collected blue mussels from several locations along the northern coast of Maine—still beyond the range of Asian shore crabs—and others deep within their southern territory. In 2002, they exposed various groups to predator signals in the lab from either green or Asian crabs, or no crabs at all.

Three months later, both the southern and the northern mussels had thickened their shells in response to the green crab, as expected. But only the southern mussels responded to the Asian shore crab. (Freeman and Byers got the same results when they repeated the experiment in the wild, with the mussels and crabs in cages off a dock in Woods Hole, Massachusetts.) This means that the southern mussels have evolved the ability to detect Asian shore crabs in perhaps as little as 15 years after first encountering them. "It's blinking fast," says Trussell, who is on Freeman's dissertation committee.

Given the many invasions under way, evolution of defenses could be quite common, says marine ecologist James Carlton of Williams College and Mystic Seaport in Mystic, Connecticut. What's novel about Freeman and Byers's research, he says, is that they happened to catch the mussels in the act. Although it's too soon to say what other evolutionary or ecological effects the Asian shore crabs might have, the finding is good news for fans of blue mussels—including those who want them on their own dinner plates. **–ERIK STOKSTAD**

CREDIT: JULIA BUCK

Now Available: H5N1 Sequences

Indonesia last week reversed itself and announced that it would put all sequence data from human H5N1 influenza patients into the public domain. Scientists say the move will help them understand how the disease is spreading.

Indonesia's samples had been sequenced by World Health Organization collaborating labs at the University of Hong Kong and the U.S. Centers for Disease Control and Prevention in Atlanta, Georgia, and placed in a password-protected influenza database at Los Alamos National Laboratory in New Mexico with limited access. Indonesia was one of several countries that resisted wider circulation of the data, a position that angered influenza researchers (*Science*, 3 March, p. 1224).

Last week, the Indonesian government reversed its position and had the password protection removed, reportedly after pressure from the Indonesian Academy of Sciences. "I'm very happy," says Ilaria Capua, an Italian bird flu researcher campaigning for broader access. "I hope this will stimulate other countries to move in the same direction."

–MARTIN ENSERINK

Taiwan Pours It On

Taiwan's National Science Council (NSC) has approved a $2.6 billion science budget for 2007 that puts the country on a trajectory to match global leaders in its level of research spending. "Being a small island without natural resources, Taiwan is in great need of R&D [for] the knowledge-based economy," says NSC's Chien-Jen Chen, an epidemiologist at Academia Sinica, the nation's top research institution. Chen has called for a 10.8% increase next year, which is expected to be adopted this fall by national legislators.

Recent research spending increases have outpaced overall governmental spending and economic growth for the past 6 years, propelling Taiwan's research investment to 3% of the country's gross domestic product by 2008. "We all feel very highly supported," says Cheng-Ting Chien, deputy director of Academia Sinica's Institute of Molecular Biology.

The new budget benefits all sectors, with special attention to mission-oriented programs in regenerative energy, earth sciences and astronomy, industrial-academic collaborations, avian and pandemic flu, and stem cells. Chen says all projects will be subject to peer review.

–DENNIS NORMILE

GENOMICS

Biofuels to Be Focus of New DOE Centers

The U.S. Department of Energy (DOE) has a reputation for bureaucratic stodginess. But last week, its science office demonstrated that it is capable of changing its mind quickly when shown a better way to proceed.

Late last year, DOE unveiled a plan to expand its genomics program from essentially a $70-million-a-year sequencing operation to a broader effort in systems biology. The key ingredient would be four large centers, each focusing on a specific area such as large-scale characterization of proteins or imaging of complex molecules. President George W. Bush requested—and Congress is set to approve—$119 million for the effort in 2007, and in January, DOE solicited proposals for the first center, which would focus on protein production.

But a month later, a panel of the National Academies' National Research Council (NRC) that had been reviewing the program for DOE sharply criticized the plan. It suggested a focus on applications, such as bioremediation or biofuels, rather than on the underlying science (*Science*, 3 March, p. 1226). In response, DOE canceled the solicitation for the first center and went back to the drawing board, a step that plant biochemist Chris Somerville of Stanford University in Palo Alto, California, called "kind of amazing."

Last week, DOE announced a new approach that hews closely to the NRC panel's recommendations. It plans to create two centers, both focused on biofuels. The centers, each funded at $25 million a year for 5 years, would use leased space, begin work quickly, and marshal multidisciplinary teams of proteomics experts, biochemists, and engineers in a friendly competition to expand knowledge of existing and emerging biofuels. Their scope would range from basic studies of microbes that digest cellulose to the development of transgenic plants that would be easier to break down and the design of new fermentation processes. DOE science chief Raymond Orbach said at a DOE advisory board meeting last week that the centers, for example, might study the metabolic secrets of the microbes within voracious Formosa termites, which break down cellulose.

Chew on this. DOE hopes its centers can learn more about how termites do their thing.

The new plan has won over critics of DOE's earlier plan. "The vertical integration is the right thing," says Somerville, who as a DOE grantee oversaw the NRC review earlier this year. "There's a reasonable expectation a lot of progress can be made" with an investment of this size in a field that has been historically underfunded, he argues.

Some bureaucrats might have tried to downplay the reversal. But Orbach says DOE's ability to change course is a sign of strength. "We completely reoriented the solicitation in 4 months and got it out," he crowed to his advisory board. White House officials encouraged DOE to place greater emphasis on energy research, say department officials, who were themselves convinced that a focused, nimble attack on specific challenges could yield results faster than a systematic attempt to tackle all the obstacles hindering genome scientists. Researchers have until February to assemble interdisciplinary teams and submit proposals, with the first awards next fall. **–ELI KINTISCH**

NUCLEAR REPROCESSING

DOE Outlines Two Roads to Recycling Spent Fuel

Six months into a Department of Energy (DOE) program to recycle spent nuclear fuel by means of an experimental method, the agency has announced plans to use more established technology to help reach its objective. Critics say the change would only exacerbate a dangerous and inefficient approach to the problem.

In February, DOE announced the Global Nuclear Energy Partnership, a central part of which was to recycle much of the 2000 tons of highly radioactive spent fuel that the U.S. produces each year. The proposed $250 million program included reprocessing facilities that would employ an experimental method called UREX+1a that breaks down used fuel into reusable chemical parts. Recycling fuel is needed to reduce a heat-buildup problem caused by waste products such as plutonium at storage facilities including the proposed Yucca Mountain repository in Nevada. The fuel recycled from UREX+1a could be burned in reactors, reducing waste and producing power.

But after months of pressure from Congress to find a quicker solution, DOE last week announced it would make $20 million available for site studies for new recycling plants and reactors. The so-called two-track approach would continue long-term studies on UREX+1a but also examine separation techniques akin to those currently in use by the French and Japanese governments. The strategy seeks technologies "that have been … in use for decades," DOE nuclear energy head Dennis Spurgeon said.

The move comes after House appropriators cut $96 million from the $243 million that DOE had requested and complained that it was "unclear why the UREX+1a process was quickly chosen as the recycling process of the future." Meanwhile, outside critics questioned whether the procedure rendered spent fuel sufficiently radioactive that potential terrorists could not safely steal it, as DOE claimed.

Princeton University physicist Frank von Hippel warns that plutonium would not be technically difficult for malefactors to separate from the kind of fuel conventional separation methods produce. DOE is abandoning "enhanced proliferation resistance in the interest of building a reprocessing plant quickly," says von Hippel, who actually prefers keeping spent fuel above ground. But DOE officials say that heightened security measures can keep recycled materials safe and that the country will benefit by the boost recycling will give to nuclear power. **–ELI KINTISCH**

CREDIT: DOE JOINT GENOME INSTITUTE

INFECTIOUS DISEASES

Gastrointestinal Virus Strikes European Cruise Ships

PARIS—You never saw passengers running for the bathroom on *The Love Boat.* But in the real world, more and more cruise vacations are being ruined by severe bouts of gastrointestinal disease. They are usually caused by noroviruses, a diverse group that causes romance-killing symptoms such as diarrhea, vomiting, and stomach cramps. This year, a network of European scientists studying food-borne viruses has already recorded 45 outbreaks on ships in European waters, which they say is a sharp increase from previous years. A similar burst occurred on U.S. ships a few years ago.

A meeting is scheduled in September at the European Centre for Disease Prevention and Control (ECDC) in Stockholm to discuss Europe-wide investigation and control strategies. In some cases, more than 40% of all passengers on a cruise have gotten ill, and several ships have experienced outbreaks on three or more subsequent trips, despite sterilization attempts in between.

Researchers aren't really sure what's behind the upsurge in norovirus outbreaks, which have become a major headache for cruise lines. Most likely, it's a result of an increased level of norovirus activity in the general population following the emergence of new strains, says ECDC epidemiologist Denis Coulombier. And cruise ships—floating minicities with ever-changing populations of hundreds or thousands of people in a confined space—are a viral mecca, just like many hospitals and nursing homes.

Spoiling the fun. Cruise ships are excellent breeding grounds for noroviruses (*inset*).

CREDITS: RICK FOWLER/REUTERS; (INSET) VISUALS UNLIMITED/CORBIS

Noroviruses can be transmitted through contaminated food, person-to-person contact (including a handshake), and contaminated surfaces such as door handles and elevator buttons. They can even become aerosolized and infect bystanders when someone throws up in public, which is why some ships have special "vomit squads" for rapid cleanup. Thorough disinfection after a trip can get rid of the virus, although crew members can also carry the virus from one cruise to the next, says Ben Lopman of Imperial College London, and new passengers can reintroduce it.

The U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, has operated the Vessel Sanitation Program since 1973, which resulted in a steady decline in norovirus outbreaks until 2000. Between 2001 and 2004, however, the number increased almost 10-fold, according to CDC researchers. Around 2002, noroviruses also began striking with increasing frequency in hospitals in Europe. In a 2004 *Lancet* paper, a large group of European researchers blamed both phenomena on a new strain within genogroup II4, the dominant group of noroviruses, that took over in 2002. The strain, they suggested, might be more virulent or more environmentally stable, or few people may have had resistance to it, leading to more widespread disease.

This year, two new strains within the same genogroup have made their debut, says virologist Harry Vennema of the National Institute for Public Health and the Environment (RIVM) in Bilthoven, the Netherlands. He suspects that noroviruses, like influenza, may evade their hosts' immune systems through frequent genetic changes, triggering fresh outbreaks along the way. That theory is hard to test, however, because, unlike flu viruses, noroviruses can't be cultured in the lab and there is no animal model.

Although cruise companies are eager to cooperate, says RIVM epidemiologist Linda Verhoef, studying outbreaks is often logistically difficult, because by the time local authorities hear about a problem, the cruise may be on its way. **–MARTIN ENSERINK**

Polish Your Stethoscopes

Singapore has spent $1 billion over the past 6 years to become a bioscience research powerhouse (*Science*, 30 August 2002, p. 1470). Now it is turning to clinical research and drug development, with a 5-year, $1.5 billion spending plan. The effort, to be vetted next week by the new Biomedical Sciences Executive Committee, draws in the Ministry of Health, extending the basic research initiative to diagnostic tests, drugs, medical treatments, and vaccines. "We will build on the basic sciences," vows Andre Wan, director of A*STAR's Biomedical Research Council. Singapore's universities and a new National Research Foundation are planning their own spending boosts.

–DENNIS NORMILE

Tax Credit Languishes

For the second time this year, Congress has failed to extend a popular tax credit meant to stimulate corporate spending on research and development. Legislators removed it from a package of tax cuts in May but promised to consider it later. But last week, Senate Republicans failed to pass a trifecta of bills that included other cuts and a higher minimum wage. The latest defeat has businesses "feeling burned," says Monica Maguire of the National Association of Manufacturers. Industry plans to try again, however, when Congress returns next month from its August recess, heartened by President George W. Bush's strong backing of the tax credit as a key to his American Competitiveness Initiative (*Science*, 17 February, p. 929). "While there's not a lot of time, it's not over," Maguire says.

–ELI KINTISCH

Gavel Falls on Biolab

A local judge wants Massachusetts and Boston University to do a more thorough review of the environmental impact of its planned biosafety level 4 lab in downtown Boston. Last week, Suffolk Superior Court Judge Ralph Gants concluded that previous assessments hadn't put enough weight on alternative sites or worst-case scenarios for the lab, which would handle highly toxic substances on Boston University's medical campus in the city's South End.

Opponents of the lab, who argued in a lawsuit that the area is too densely populated for such biological work, were elated. But the decision won't halt construction of the $178 million building, the future of which, Gants noted, the ruling does not address. The university intends to appeal.

–ANDREW LAWLER

MOLECULAR IMAGING

New Optics Strategies Cut Through Diffraction Barrier

Optical microscopes gave birth to cell biology, revealing a Lilliputian world of mitochondria, chromosomes, and much more. Yet as biologists grew more adept at illuminating the cell's interior, light's physical properties stopped their progress dead in its tracks. The so-called diffraction barrier limits resolution to 200 nanometers in the case of visible light, or half the wavelength used to make an image. To see more detail, scientists had to turn to the shorter wavelengths of electron microscopes.

Now, two research teams have independently developed light microscopy techniques that resolve objects on the nanometer scale. "The diffraction barrier is not only gone in theory. It's really gone," says physicist Stefan Hell of the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, the leader of one of the groups. He and others expect the new methods to enable biologists to visualize how proteins interact with one another and the cell membrane, and to solve numerous mysteries about how cells function. "I see a whole array of applications," says Shuming Nie, a biomolecular engineer at Emory University in Atlanta, Georgia.

One of the new techniques, described online in *Science* this week (www.sciencemag.org/cgi/content/abstract/1127344) by physicists Eric Betzig, Harald Hess, and colleagues, began with a device assembled in Hess's living room while both he and Betzig were unemployed. Betzig had pioneered a technique called near-field microscopy at Bell Labs in the 1990s, but he then went to work at his father's machine tool company in Michigan. "I was going through my midlife crisis, [and] I didn't want to do microscopy," says Betzig. Leaving the machine company in 2003, he began talking microscopy again with Hess, a longtime friend from Bell Labs.

Up close. A high-tech microscope, assembled in a living room (*above*), revealed molecules (red, *inset*) nanometers apart inside a cell's mitochondria.

Together, the two arrived at a way to break the diffraction barrier. Using new technologies for labeling cellular machinery with light-activated fluorescent markers, they could "turn on" just one molecule at a time. Such pinpoints of light can be located much more precisely than when all are glowing at once. By slowly mapping the cell molecule by molecule, they could piece together a high-resolution picture of the whole thing.

They constructed a microscope that flashes a violet light at proteins designed to activate under such rays. By keeping the light ▶

CAREER DEVELOPMENT

NSF Wants PIs to Mentor Their Postdocs

U.S. funding agencies have traditionally steered clear of micromanaging the relationships between principal investigators (PIs) and their postdocs, although federal grants typically pay the salaries of these unsung lab heroes. Postdocs say this hands-off policy encourages PIs to treat them as skilled laborers rather than apprentice scientists. Last week, the National Science Foundation (NSF) took a small step toward addressing that complaint with a directive aimed at getting scientists to take their mentoring role more seriously.

A 2 August letter from the agency's geosciences directorate asks grantees and grant applicants to spell out their mentoring activities in both grant proposals and annual and final reports (www.nsf.gov/pubs/2006/nsf06038/nsf06038.jsp). The goal, say NSF officials, is to make sure that postdocs acquire vital skills such as grant writing, lab management, research ethics, and teaching at the same time they are advancing the frontiers of science. The words are more of a carrot than a stick, says Jim Lightbourne, a senior adviser in the agency director's office, who says he hopes the initiative "will serve as a model for other NSF directorates."

The letter asks that PIs report specific training efforts and describe their impact. NSF is particularly interested in "highly effective or innovative ways" of molding the next generation of scientists, notes geosciences head Margaret Leinen, who took the idea from a 2004 NSF workshop on postdoc training. Leinen's letter includes a none-too-subtle reminder that such activities fall within the scope of one of the two criteria used to judge grant proposals.

Although the letter does not mandate mentoring, it's "an important first step" toward making PIs more accountable, says Alyson Reed, executive director of the National Postdoctoral Association (NPA). "We still hear stories of PIs discouraging their postdocs from attending workshops because it'll take time out of their day," she says. NPA plans to press the other NSF directorates and the National Institutes of Health to adopt similar guidelines.

Giuseppe Petrucci, a geochemist at the University of Vermont in Burlington, would have liked to see NSF use more forceful language: "Right now, it merely reads like a suggestion that grantees can easily ignore." The problem, says Petrucci, an assistant professor, is that "academic researchers understand that graduate students need to be trained. But they take postdocs as being independent. It's difficult to change that mindset."

–YUDHIJIT BHATTACHARJEE

CREDITS: HARALD HESS; (INSET) E. BETZIG *ET AL.*, *SCIENCE*

flash brief and the light extra dim, the scientists ensured that just some molecules activate. Then, the pair zapped the molecules with a yellow light that made them glow brightly for up to a few seconds before flaring out. By repeating the process over and over again—roughly 10,000 times in all over 2 to 12 hours—the researchers could gather enough information to compile a "supermap" of the cell, distinguishing molecules just 2 to 25 nanometers apart in regions with up to 100,000 molecules per square micrometer. For example, they assembled detailed images of the Golgi apparatus and the retroviral protein Gag bound to the cell's membrane. "They are, in a sense, pushing the power of single molecules as nanoscale light sources to the limit," says W. E. Moerner, a physical chemist at Stanford University in Palo Alto, California.

The new technique, dubbed photoactivated localization microscopy, currently has a resolution similar to that of electron microscopy. But scientists say that it has potential for even better resolution and for examining protein-protein interactions, particularly if fluorescent labels of different colors can be applied to proteins.

Hell's barrier-busting technique, which he first sketched out in 1994, takes the opposite approach from Betzig's. Instead of turning on fluorescently labeled molecules one by one, Hell turns them off, using a hollow needle of light that darkens a ring of molecules but leaves the ones in the very center glowing. In 2000, Hell tested the technique—known as stimulated emission depletion microscopy—on cells and found that it worked. Last year in *Physical Review Letters*, Hell and colleagues reported even better resolution in nonbiological samples. Now, in the 1 August *Proceedings of the National Academy of Sciences*, Hell and colleagues report imaging molecules 15 to 20 nanometers apart in dead cells.

One challenge now is to apply the new techniques to living cells, whose parts are often in rapid motion. The Betzig technique may face more hurdles because it relies on hours of snapshots before building a picture of a cell's static state. Still, says Moerner, there's hope that scientists will find ways around the roadblocks. "The ingenuity of people always surpasses what we say can be done," he says.

Fortunately, Hess's living room won't be needed anymore. Both Hess and Betzig have been recruited to lead groups at Janelia Farm, the new Virginia campus of the Howard Hughes Medical Institute devoted to developing new research techniques.

–JENNIFER COUZIN

ASTRONOMY

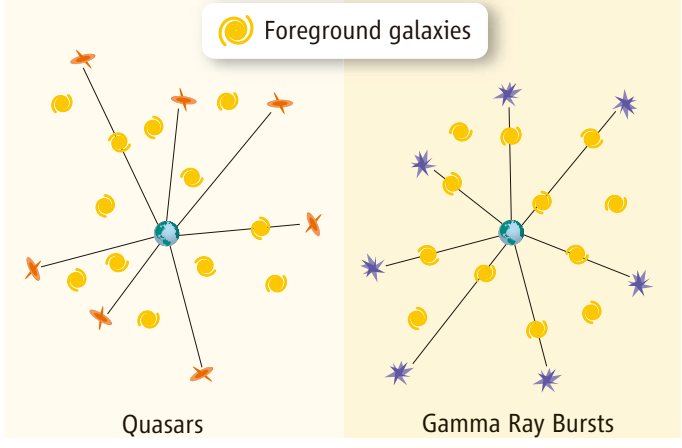
Do Gamma Ray Bursts Always Line Up With Galaxies?

Astronomers studying gamma ray bursts (GRBs) have stumbled upon a mystery. Apparently, these hugely energetic explosions in the distant universe prefer to go off in places where at least one galaxy lies between them and Earth. But quasars, which are also very remote, don't share that preference—and nobody can explain why. "It's a very puzzling result," says Krzysztof Stanek of Ohio State University in Columbus.

Earlier observations of thousands of quasars (the luminous nuclei of distant galaxies) showed that about a quarter of them bore the spectroscopic fingerprints of foreground galaxies. But when a team led by Jason Prochaska and Gabriel Prochter of the University of California, Santa Cruz, did the same analysis for 14 GRBs with known distances, they found one or more foreground galaxies in almost every case. In a paper accepted for publication in *Astrophysical Journal Letters*, they describe the find as "astonishing."

Prochaska and his colleagues have studied several possible explanations for the find. Dust absorption in the foreground galaxies might be different for quasars and GRBs, in ways that obscure more quasars. Large-scale gravitational lensing by the intervening galaxies might boost the brightness of GRBs and so make them easier to detect. Finally, the galaxylike features in the GRB spectra might come from the "home galaxy" of the burst, not a foreground galaxy. But, says cosmologist Martin Rees of Cambridge University in the U.K., "as the authors themselves realized, none of their suggested explanations works very well."

In an as-yet-unpublished paper, however, a team of Ohio astronomers including Stanek and Stephan Frank claim they can explain Prochaska's result in a particular set of circumstances: if the gas in the foreground galaxies is clumpy and the light-emitting region of a quasar is bigger than the corresponding region of the fireball of a GRB. Stanek concedes that this is just the reverse of common astrophysical wisdom, but, he says, "it's at least a plausible explanation that should be looked into." However, the Ohio proposal has met with quite a bit of criticism, says Frank.



Spooky, or what? Light from most gamma ray bursts seems to pass through a galaxy en route to Earth, unlike light from similarly distant quasars.

Could the result be due to chance? After all, the number of GRBs used in the study is relatively small. Ken Lanzetta of Stony Brook University in New York thinks so. "If I had to bet, I would say this is that one-in-10,000 statistical fluke that happens every now and then," he says. "It will probably go away when more observations become available. We'll have to wait and see."

If the puzzle remains after 15 or 30 more GRBs are analyzed, however, then "something very strange must be going on," Lanzetta says. But cosmologist Jeremiah Ostriker of Princeton University is confident that a solution will be found. Meanwhile, Prochaska says he would welcome any suggestions. "I'm desperate enough to consider out-of-the-box ideas," he says. "I'm stuck at the moment."

–GOVERT SCHILLING

Govert Schilling is an astronomy writer in Amersfoort, the Netherlands.

A radical new interpretation of string theory raises the prospect of untold numbers of separate universes with different physical laws—an idea that some physicists say threatens the foundation of their science

A 'Landscape' Too Far?

NEWPORT BEACH, CALIFORNIA—Physicists have long heaped scorn on anyone who tried to explain features of the universe by pointing out that had they been otherwise, life would be impossible.

This "anthropic principle," many physicists charged, abandoned the long-standing goal of finding equations that specify all of nature's properties. Most preferred the notion that a comprehensive theory would account for everything the universe has to offer.

Ironically, however, the favored candidate for that approach—superstring theory—may be exacerbating the very problem everybody hoped it would solve. Far from disposing of anthropic reasoning, string theory has reinvigorated its advocates, leading to a philosophical schism within the physics community.

The dispute has touched off sharp exchanges both within and outside science journals. In January, for example, experimental physicist and Nobel laureate Burton Richter of Stanford University in Palo Alto, California published a letter in the *New York Times Book Review* blasting the anthropic approach as sterile and unscientific. Its proponents "have given up," he wrote. "I can't understand why they don't take up something else—macramé, for example." Another Nobel laureate, David Gross of the Kavli Institute for Theoretical Physics at the University of California, Santa Barbara (UCSB), compares anthropic thinking to a disease. "I inoculate myself by emotional intensity against it because it's very contagious," he says.

> **"I inoculate myself by emotional intensity against [this idea],** because it's very contagious."
>
> —David Gross, UC Santa Barbara

On the other hand, Stanford University physicist Leonard Susskind believes that anthropic reasoning may be the wave of physics' future. Susskind is a leading advocate of a new view of reality called the superstring landscape, in which the known universe is just a tiny habitable corner of a grander reality. If the landscape idea is correct, string theory offers no specific predictions about the universe's properties but rather implies the possible existence of a countless number of combinations of properties—like a vast landscape with differing physical features.

In the landscape scenario, life can exist only where the mix of properties leads to a hospitable environment—precisely the sort of reasoning long used by advocates of the anthropic principle. So the string landscape has emboldened many supporters and even converted some skeptics into saying the a-word aloud—much to the dismay of its die-hard opponents.

During a panel discussion at a recent physics conference here,* Richter recited a blistering indictment of the landscape and its anthropic implications. "The anthropic

* 14th International Conference on Supersymmetry and the Unification of Fundamental Interactions, 12–17 June.

CREDITS: (PHOTOS) GETTY IMAGES; NASA; (PHOTO ILLUSTRATION) K. BUCKHEIT KRAUSE/*SCIENCE*

principle is an observation, not an explanation," he declared. "The landscape, as far as I can see, is pretty empty. … It looks to me that much of what passes for theory these days is more like theological speculation."

Views like those expressed by Richter and Gross have dominated physics for decades, with anthropic reasoning relegated mostly to pub discussions and the occasional popular book. But that began to change around the turn of the millennium, when the supposed cure for anthropic reasoning—superstring theory—suddenly began to spread the disease.

Throughout the 1980s and '90s, superstring theory was frequently advertised as potentially being the ultimate "theory of everything." By conceiving basic bits of matter as loops or snippets of string, rather than tiny points, superstring theory offered the prospect of merging general relativity and quantum mechanics into a consistent framework. Its supporters hopefully predicted that the final version of string theory would precisely specify all of nature's features as natural outcomes of some master equations.

But in 2000, Joseph Polchinski of UCSB and Raphael Bousso, now at UC Berkeley, published a landmark paper in the *Journal of High Energy Physics* (*JHEP*) that put the landscape on the string-theory map. Technically, they showed that the theory permits a huge number of different metastable vacuum states—that is, spaces that could exist for a long time with a vast range of physical properties, such as the masses of basic particles and the density of energy in the vacuum of space.

For years, theorists have struggled in vain to calculate the density of the vacuum energy, now known commonly as the "dark energy" thought to be driving the universe's accelerating expansion. But their calculations give an answer that is too high by something between 10^{60} and 10^{120} orders of magnitude.

If the string landscape exists, however, the problem is moot. In the landscape, the vacuum energy can take on all sorts of possible values. If there is no one right answer for the vacuum energy's value, that could explain why no theory could predict what it is. Physicists are around to ponder the issue only in a space where the vacuum energy's value permits life to exist.

In the landscape story, the local amount of vacuum energy is an environmental accident that happens to permit life's existence rather than a natural outcome of basic laws of physics. But determining what is "natural" in physics is itself a contentious issue. At the Newport Beach conference, a panel session convened to discuss "naturalness" became a forum for debating anthropic reasoning.

> "Much of what passes for theory these days is **more like theological speculation.**"
>
> —Burton Richter, Stanford University

Susskind pointed out that the string landscape meshes nicely with developments in big bang cosmology since the early 1980s, when Alan Guth, Andrei Linde, and others developed the theory of inflation. In that view, a tiny patch of space burst suddenly larger in a brief instant of inflationary expansion; the newborn universe then continued expanding at a more leisurely pace to produce the mature universe observed today. Satellite observations have provided strong support for inflation's predictions about features imprinted in the cold glow of microwaves left over from the big bang.

If the inflationary origin of the known universe is correct, the same process could have happened over and over again, with new "bubble" universes forming within old ones ad infinitum—a scenario known as eternal inflation. In the early 1980s, Linde, Soviet physicist Andrei Sakharov, and others pointed out that the resulting "multiverse" of bubbles might explain certain mysteries anthropically. Each bubble might have a different density of vacuum energy, some very high. But a large vacuum energy makes galaxy formation (and hence stars, planets, and people) impossible. Our bubble must therefore have a small vacuum energy—possibly zero—in order for life to exist.

In 1987, that argument was made more precise by Steven Weinberg of the University of Texas, Austin, another Nobel physics laureate. Weinberg showed that the existence of life did not require that the vacuum energy be zero, only that it be much smaller than physicists had calculated. About a decade later, evidence for cosmic acceleration bore out the prediction of nonzero vacuum energy in our universe.

Adversaries. In a panel discussion on the nature of physical law, theoretical physicists Andrei Linde (*left*) and Burton Richter disagreed sharply about the status of the "string-theory landscape."

CREDIT: T. SIEGFRIED

Most string theorists initially ignored the discovery of vacuum energy—or assumed their theory would eventually explain its magnitude, whatever it was. Around that time, Polchinski began discussions with Bousso about string theory's relation to cosmology. By 2000, they had produced the *JHEP* paper suggesting that string theory itself forecast an incredible number of possible vacuum states (by current estimates, perhaps as many as 10^{500}, or even more).

Changed man. Joseph Polchinski once told a colleague he'd quit physics rather than invoke the anthropic principle.

A Reluctant Convert

Like most physicists, Joseph Polchinski never much liked the idea that the existence of life had anything to do with the nature of the universe.

The so-called anthropic principle—that properties of the universe were somehow inexplicably hospitable for the evolution of life—smelled too much like metaphysical mush. Physics was supposed to find equations that answered basic questions about the cosmos, such as how much energy resided in the vacuum of space.

But the equations predicted far too much vacuum energy to allow the formation of galaxies or any conceivable habitat for life. So most physicists thought there simply was no such energy—that the vacuum energy, technically known as the cosmological constant, was zero. If it were not zero, but still small enough to allow life, it would be hard to see how to explain it with equations. In fact, Polchinski told cosmologist Sean Carroll a decade ago, if astronomers ever found evidence for a nonzero cosmological constant, he'd give up physics—because that would signal the need to invoke the anthropic principle.

Such a vast repertoire of possible universes emerged from the many convoluted ways in which the objects of string theory can twist themselves up. String theory's hallmark (and to some, most horrifying) feature is its need for six or seven extra dimensions of space beyond the three dimensions of ordinary experience. One-dimensional strings vibrate within this higher dimensional space, with different modes of vibration corresponding to different kinds of particles. Other objects can exist, such as two-dimensional "membranes" and other "branes" of higher dimension. String theory analogs of magnetic fields (called fluxes) can emanate from the branes. And string theory's multiple dimensions fold up on themselves in thousands of configurations containing spacetime gaps (or handles) sort of like the hole in a doughnut. The universe's physical properties depend on the resulting arrangement of the strings, branes, fluxes, and handles, and they can assume a nearly countless number of configurations. Just as protons, neutrons, and electrons can combine to produce hundreds of atoms and thousands of molecules, Polchinski says, branes, handles, and fluxes can produce a vast number of different species of spacetime.

At first, many physicists dismissed string-landscape vacuums as quirks of the math with no relation to reality. But in 2003, a paper by Linde, of Stanford, and three collaborators (Shamit Kachru, Renata Kallosh, and Sandip Trivedi) published in *Physical Review D* showed that the many vacuums in the landscape might actually exist, at least long enough to give rise to life.

Since then, the landscape concept has generated a burgeoning bibliography of papers along with relentless antianthropic animosity. Anthropic explanations "are fun parlor games," says Gross, director of the Kavli Institute for Theoretical Physics at UCSB. "But they're not science in the usual sense of making predictions that can be tested to better and better precision over the years."

Gross fears that anthropic infections might incapacitate attempts to find unique answers to tough questions by inducing people to give up the quest. He cites historical examples in which seemingly incalculable features of nature—say, the spacing of energy levels in atomic nuclei—eventually yielded to reductionist explanation. In fact, he emphasizes, nearly all the normal, observable world can in principle be explained by the standard model of physics without resorting to any anthropic considerations. "Most people have absolutely no idea how successful science has been at explaining, with one or two parameters, all of the physics that they know of in everyday life," he said.

Richter expresses similar sentiments. "I don't see any problem with part of the theory community going off into a metaphysical wonderland, but I worry that it may be leading too many of the young theorists into the same thing," he says.

Landscape advocates reject such criticisms, contending that opposition to anthropic reasoning is largely emotional. "There's no substantive scientific debate," Susskind says. "The nature of what is going on is different emotional reactions to some facts and some interpretations of those facts that we've discovered." And those facts suggest that the universe is vastly larger than what scientists can see.

"We no longer have any evidence that our little piece of the universe is representative of the whole thing," Susskind argues. And if the universe is not everywhere the same, then the properties of nature that physics has tried to specify would differ from place to place. "Once we agree that it's diverse, then some features of it are environmental," he says. "We have to figure out which ones."

But that doesn't mean that physics must abandon the goal of making testable predictions. "We're all struggling quite hard to make observational physics out of it," Susskind says. And Linde points out that future observations of gravitational waves from the early universe could falsify, or verify, anthropic predictions about the nature of spacetime curvature predicted on anthropic grounds.

"It's science," Linde asserted during the Newport Beach panel discussion. "It's not science fiction. It's not religion. … It's something where we can really use our knowledge of mathematics and physics and cosmology." Far from taking the easy way out, as its opponents sometimes allege, anthropic science is depressingly difficult, he observed. "It's complicated. It's not an easy job to do, so if you don't

CREDIT: LUBOS MOTL

As a leading superstring theorist, Polchinski, of the Kavli Institute for Theoretical Physics at the University of California (UC), Santa Barbara, was in the thick of the fight to find the ultimate equations describing reality, the somewhat mislabeled "theory of everything" that should have unified gravity with nature's other forces. "People in string theory were very fixed on the idea that there was some powerful mathematical structure we hadn't fully identified, and when we did, we would know why the cosmological constant was exactly zero," recalls Polchinski.

Even in 1997, when astronomers reported evidence for a nonzero cosmological constant, "very few string theorists either knew or wanted to admit the significance of it in terms of the anthropic principle," Polchinski says.

Neither did Polchinski himself. But shortly thereafter, he began a collaboration with Raphael Bousso, now at UC Berkeley, that led to a shocking result: String theory itself predicted numerous possible vacuum states with different values for the cosmological constant. Dismayed by the anthropic implications, Polchinski was reluctant to publish the results, but Bousso insisted. "We totally agreed on the science," Polchinski says, "but he was the one who really said, 'Look, we've got to publish this.' "

After the paper was published in the *Journal of High Energy Physics* in 2000, Polchinski remained in quasi-denial, unwilling to embrace the anthropic "dark side" of physics. But the paper inspired others to investigate what came to be called the "landscape" of vacuum-state possibilities. Most outspoken among them was Leonard Susskind of Stanford University in Palo Alto, California.

"Lenny came along and said, 'Look, we can't sweep this under the rug; we have to take this seriously,' " Polchinski says. "If this is the way things are, science is only going to move forward by thinking about it, not by pretending it's not there."

However reluctantly, Polchinski has now become an anthropic advocate of sorts. His tipping point, he says, came at a dinner for donors to the Kavli Institute. One attendee asked about the anthropic principle.

"And I said nobody believes that," Polchinski recalls. "And when I said that, I knew I was lying. I knew that the evidence was mounting for the anthropic principle."

So 2 years after the landscape paper appeared, Polchinski delivered his first talk on the topic, describing the landscape and acknowledging its anthropic implications at a conference in Chicago, Illinois. Carroll, of the University of Chicago, was there, Polchinski remembers: "He immediately said, 'Can I have your desk?' "

–T.S.

want to do it, then don't do it. But don't say that it's not science."

Other physicists, although reluctant to embrace anthropic reasoning, decry the acrimony and seek a middle ground. "It's unfortunate that it has turned into a situation where you have to choose to be in one camp or the other," says Clifford Johnson, a string theorist at the University of Southern California in Los Angeles. "It would be nice if we could explore some of those unpalatable ideas just in case that's the way that nature chooses to go."

Of course, it's possible that the landscape will turn out to be wrong. "It may well be that further understanding of string theory will show that the multiple possible spacetime vacuums are just phantoms," Johnson says.

Nobel laureate Frank Wilczek of the Massachusetts Institute of Technology in Cambridge, another speaker at the Newport Beach panel, agrees that the fate of the landscape idea remains uncertain. "I don't think the landscape is established to any convincing level of rigor," he says. "There are lots of shaky aspects to the argument."

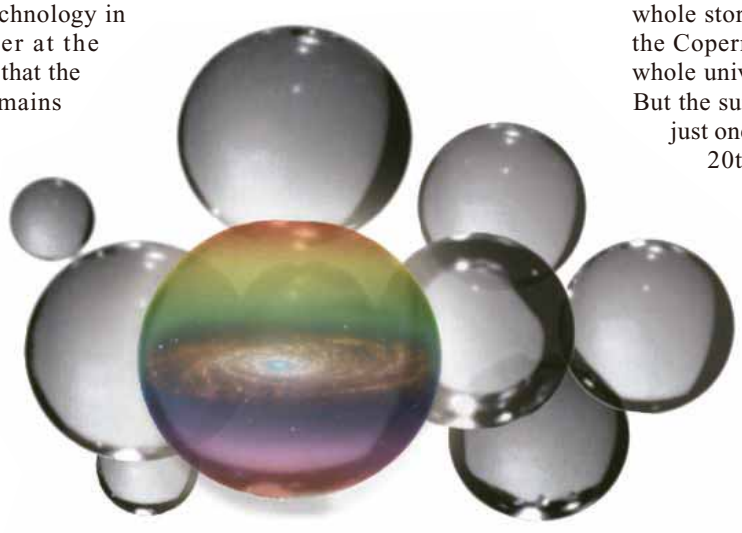
In fact, technical objections to the reality of the landscape have been raised, notably by Tom Banks of UC Santa Cruz. And recent work by Paul Steinhardt of Princeton University and Neil Turok of Cambridge University in the U.K. suggests that the vacuum-energy problem can be explained "naturally," without anthropic reasoning, if the universe undergoes a cyclic repetition of expansion and collapse. Recent work by Stephen Hawking of Cambridge University and his collaborator Thomas Hertog of CERN, the European particle physics laboratory near Geneva, Switzerland, suggests that rather than describing a multiverse of spacetime bubbles, the landscape reflects alternative realities embodied in the equations of quantum mechanics. Under Hawking and Hertog's assumptions, only a few of the landscape's realities have a significant probability of actually existing.

"It's not an easy job to do, so if you don't want to do it, then don't do it. **But don't say that it's not science."**
—Andrei Linde, Stanford University

Given the current state of knowledge, efforts to either confirm or refute the landscape's anthropic implications are simply premature, says cosmologist Sean Carroll of the University of Chicago in Illinois, who will soon be moving to the California Institute of Technology in Pasadena. But, he says, the idea that the known universe is only a small part of something much bigger should not come as so much of a shock. "Again and again in the history of cosmology, we've been shown that the little pieces we've been looking at are not the whole story," Carroll says. At the time of the Copernican revolution, the supposed whole universe was just the solar system. But the sun eventually was revealed to be just one star in a vast galaxy, and in the 20th century, that galaxy became just one speck in space among billions and billions of others.

As Wilczek observes, the string landscape and the multiverse merely suggest that the same story is happening again. "This is going one step further," he says. "We should be used to it by now."

–TOM SIEGFRIED



CREDIT: (PHOTO ILLUSTRATION) K. BUCKHEIT KRAUSE/*SCIENCE*

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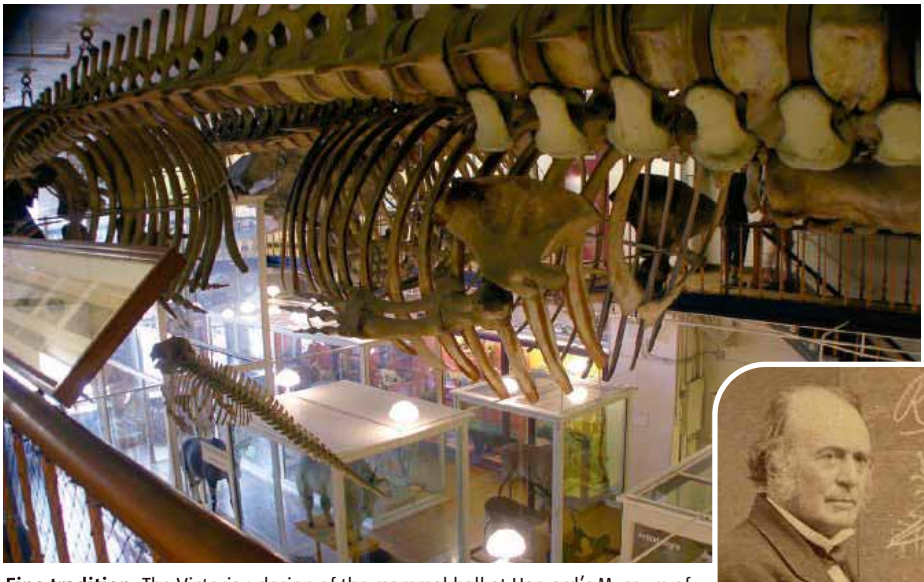
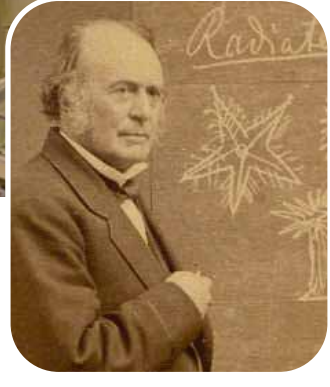
Shaking the Dust off Agassiz's Museum

As its director, Jim Hanken has gone to bat for the Harvard Museum of Comparative Zoology, scoring space for its collections and more say in organismal biology

WEST TOWN, MASSACHUSETTS—Searching out salamanders is a bit like divining for water, and Jim Hanken and his students know just where to look. Within 30 minutes of peeking under rocks and pulling apart decaying logs at a state forest 70 kilometers outside Boston, the Harvard-based crew has found every common species of salamander in the area.

Today, however, they are searching for eggs belonging to *Plethodon cinereus*, the red-backed salamander. Most amphibians spend their youth as larvae in streams and ponds, but this salamander hatches four-legged, terrestrial young directly from eggs. Hanken's group wants to learn how this difference evolved by comparing *P. cinereus* to a closely related species with aquatic larvae.





Fine tradition. The Victorian design of the mammal hall at Harvard's Museum of Comparative Zoology is one of the legacies of the founder, Louis Agassiz (*inset*).

But on this day, the researchers go home disappointed. They will need to come back in a few weeks and search again for the eggs.

Disappointment is nothing new to Hanken; his 25 years of studying amphibians and reptiles have taught him to be patient. For decades, he's been collecting a group of tiny Mexican salamanders. "Most of the time, we go for long stretches and get nothing," says Hanken, a vertebrate morphologist. But "we have to tell ourselves that we're in for the long haul."

Persistence has served Hanken well as director of Harvard's Museum of Comparative Zoology (MCZ), one of the world's top university natural history museums. Since he took over in 2002, Hanken has been pushing, against some resistance, to revitalize the museum's physical space and make sure its extensive collection of 21 million specimens has a secure future.

At the same time, he has been trying to guarantee that MCZ is a central player in biology on campus and in the international museum scene. "That's a big job," says Scott Edwards, MCZ curator of birds. He and others welcome Hanken's energy. Yet some are bothered by changes in the way MCZ is set up and operated. "It's more corporate under Jim," says entomology curator Brian Farrell.

When Hanken took over as MCZ's director, organismal biology at Harvard, and particularly at MCZ, were at risk of getting left behind as Harvard hustled to power up genomics, stem cell science, systems biology, and other "hot" areas of biology. Hanken scrambled to close ranks with other evolutionary biologists on campus to boost the museum's presence and keep the evolutionary biology program strong.

Getting the rank and file to sign up wasn't so hard; getting the attention of then-president Lawrence Summers was very difficult. Summers "hated museums," Hanken says, considering them outdated. The university was eyeing MCZ space for its own purposes, and there was talk of moving the collections off campus. Summers felt "we shouldn't have the museum any longer," Hanken recalls. Founder Louis Agassiz must have been turning in his grave.

Now Summers is gone, having stepped down on 30 June. Renovations are under way at MCZ, and new curators are setting up shop. Although some collections are still moving out, they will, most likely, end up in a new building right next door. Some curators have signed on to Hanken's plan, whereas others have adopted a wait-and-see attitude, Hanken says. And he's betting that patience will pay off.

Keeping up with the times

Hanken accepted a job at MCZ in 1999 as the herpetology curator; 3 years later, he became MCZ's director (see sidebar, p. 755). Since then, he's tried to integrate the museum more closely with Harvard and with the biodiversity movement.

The museum has been moving from total autonomy to limited independence, a process set in motion 30 years ago by former director Fuzz Crompton, who pushed to have MCZ curators appointed as Harvard faculty. (Few had a connection before that.) MCZ continued to set its own course virtually independent of Harvard for a time. Now, issues of space, faculty hires, and future directions are decided jointly by MCZ and Harvard's biology units. That "does tie our hands," says Hanken, but there is an important quid pro quo: MCZ has a louder voice in the future of biology at the university.

Hanken is building up core facilities and has hired staff to standardize managerial work. He plans to have a centralized collections database and frozen tissue depository. This has translated into more meetings, more forms to fill out, more people to go through when collecting permits are needed, and "a whole new raft of [government] regulations," says vertebrate paleontology curator Farish Jenkins. "It's been harder to be productive with your research."

Even a casual observer notices the changes at MCZ. When Hanken arrived at the museum 7 years ago, he was struck by how rundown it was. Floors sagged; the roof

CREDITS: JON WOODWARD/MCZ; (INSET) ERNST MAYR LIBRARY, MCZ

An Outsider Moves In

Harvard University is known as a tradition-entrenched institution, and the Museum of Comparative Zoology (MCZ) may sit in one of the deepest trenches. Its halls echo with the footsteps of giants such as museum founder Louis Agassiz and famous leaders such as the late evolutionary biologists Ernst Mayr and Stephen Jay Gould. Typically, MCZ has hired from within the Harvard community, but not when it chose James Hanken, its current director. Hanken came from outside—indeed, he had even been rejected by MCZ for graduate school in 1973. But he's an insider now, and he seems right at home.

Antique microscopes sit on Hanken's bookshelf. Illustrations of skulls fascinate him, too; on his wall are postcards of famous paintings with subjects holding or standing by human skulls. He says he'd like to write an art history book on them one day. As a postdoc in the early 1980s, he helped bring back into print a classic text from the 1930s, *The Development of the Vertebrate Skull* by Gavin de Beer. Later, Hanken and his adviser, Brian Hall of Dalhousie University in Halifax, Canada, followed in de Beer's footsteps and put together a new three-volume modern treatise on skulls, the Bible of cranial development, according to Timothy Carl, a former Hanken student and now a biotech strategy consultant.

A New York City native, Hanken got hooked on the natural world reading a Time-Life series on animals. "I would devour those books," he recalls. He studied zoology at the University of California, Berkeley, and spent a summer tagging sea turtles. He was drawn to the new discipline of conservation biology, but, unable to find a lab position in this field, he moved into a more traditional one, systematics, with Berkeley's David Wake, a herpetologist.

Modern modules. Hanken replaced the MCZ's "Agassiz drawers."

Wake sent Hanken far afield to Mexico to make sense of a genus of salamanders called *Thorius*, which proved to have more species than anyone anticipated, many of which were hard to tell apart. One thumbnail-sized species caught Hanken's eye. His studies on the evolution of skull parts in these tiny animals "focused [researchers'] attention on the skull as modular, [with] evolution in one region occurring independent of changes in other regions," says Hall.

During this time, Hanken flirted with the idea of becoming a nature photographer. Today, his office has a wall of framed magazine covers exhibiting his photos. His agent warned him how hard it was to make a career as a photographer; Hanken stayed in biology.

He arrived in Nova Scotia in 1981, where he learned developmental biology, which took him to a job at the University of Colorado, Boulder, 2 years later. Hanken's keen eye for morphological differences among closely related species helped him key in on how genetic changes reshaped jaws or limbs, for example. By looking for these changes in the salamander family tree, among others, he and his colleagues gained insights into the genetic underpinnings of evolutionary change. His approach, says Scott Gilbert, a developmental biologist at Swarthmore College in Pennsylvania, has helped stimulate a growing discipline called evo-devo in which evolutionary and developmental biologists swap ideas and techniques to understand the evolution of complex organisms. **–E.P.**

leaked. The facility lacked modern amenities such as local parking or adequate restrooms. "At one time, the museum was state of the art, but now [the building] was simply not adequate," Hanken recalls.

He installed new lighting in herpetology collections, painted the walls and ceilings white, and swept away the dark, dingy appearance. In what some viewed as a brash innovation, Hanken replaced the famous "Agassiz drawers"—designed by Louis's son Alexander Agassiz more than a century ago—with movable, pest-proof cabinets, increasing the storage capacity for herpetology by 60%.

As director, he's taken aim at speeding up the modernization of the rest of the museum. The first order of business was the overcrowded collections. "We've got 10 million mollusks tucked into every crack in the wall," complains former director Jim McCarthy, a biological oceanographer. Even the attic is crammed full of large mammal bones, including a fully articulated killer whale hanging from the ceiling.

Some curators were convinced nothing less than a new building would do, with space for collections and curators' labs and offices. The university instead proposed moving just the collections off campus. But specimens would have been too inaccessible for teaching and research, Hanken points out. After several years of discussion, that idea was scrapped. Hanken has been campaigning for space in the life sciences building going up outside his window. It would enable the collections to grow relatively unimpeded, he says.

The university listened: If all goes as proposed, four of the 10 collections, including birds and mammals, soon will be hoisted from MCZ's upper floors and lowered into 2900 square meters of underground storage in the new building. It's not the ideal solution but will suffice for quite a while, says Hanken. But it has "been a tough issue" for some curators, says Edwards. He and his colleagues are used to pulling out a specimen from across the room. Soon they may have to take a roundabout route via stairs and corridors to the new building.

Hanken and the university are also discussing converting public display areas to other uses. The building's third floor is a labyrinth of cases filled with stuffed animals. There is even a Victorian gallery of mammals that looks much like it did when it was first set up shortly after MCZ was founded in 1859. "It's a museum of museums. It's kind of musty and old-fashioned, but I think of it as a fabulous treasure," says entomology curator Naomi Pierce. There's talk of moving the displays to a new campus across the Charles River and amalgamating them with art and exhibits from other Harvard museums. It would free up space, but Pierce is wistful about the museum leaving the building.

Yet she and her fellow curators appreciate where Hanken is coming from. "Jim has tried to move the museum from the 19th century to the 21st century," Edwards points out. And Hanken is convinced he will succeed. "In a few years," he says, "the MCZ will be completely transformed."

–ELIZABETH PENNISI

CREDIT: E. PENNISI

Up, up, and away. Artists' conceptions of stratospheric airships designed for telecommunications (*right*) and optical astronomy (*below*).

ASTRONOMY

Low Road to the Heavens

Aficionados say balloon-borne observatories could rival the power of space telescopes at a small fraction of the cost

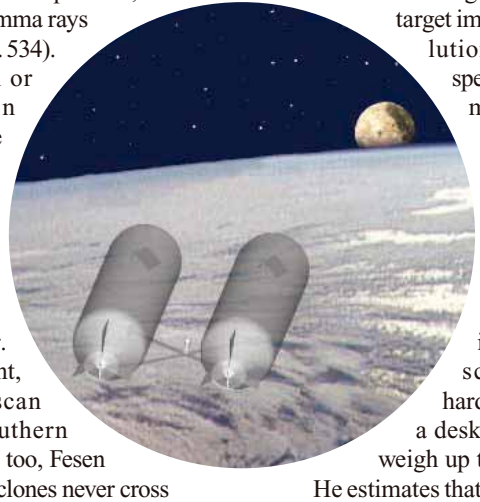
If Robert Fesen gets his way, the next generation of telescopes that are launched into the sky might never reach space.

Instead Fesen, an astronomer at Dartmouth College in Hanover, New Hampshire, wants NASA to consider stationing optical telescopes on high-flying lighter-than-air craft. Crewless airships could do much of what the Hubble Space Telescope does but at a fraction of the cost, Fesen says. Perched above both the weather and 95% of Earth's atmosphere, under dark skies even during the day, the telescope could scan the heavens with an acuity limited only by the size of its mirror and the quickness of its pointing apparatus.

If technological roadblocks can be successfully navigated, Fesen says, such "Hubble Juniors"—projected to cost between $5 million and $10 million apiece—could be within budgetary reach of many nations and scientific consortiums that cannot afford their own space telescopes. Such airship observatories, able to retarget to anywhere in the sky within minutes, would be well suited for studying supernovae, for instance. Today, precious hours after a supernova's discovery can be wasted locating and waiting for ground- or space-based telescopes. Fesen's paper setting out the proposal is now online on the arXiv.org preprint server and appeared in June in the *Proceedings of the International Society for Optical Engineering*.

"There's a lot of people sensing that the only way to make a better telescope is to make a bigger telescope," says James C. Green, an astronomer at the University of Colorado, Boulder. "But they don't think, 'Let's go for a completely new way of doing things.' So it meets a little resistance initially." But Fesen's proposal is "exciting," he says. "It's feasible, and it's doable."

Since the first balloon-borne meteorological studies in the 1860s, lighter-than-air craft have carried science skyward. Nearly a century later, Martin Schwarzschild's Stratoscope instruments captured pioneering high-resolution images of the sun, outer planets, and galactic nuclei from an altitude of nearly 25 km. And today, balloons such as the BOOMERanG cosmic background observer venture into the stratosphere to gather astronomical and cosmological data across the spectrum, from microwaves to gamma rays (*Science*, 28 July 2000, p. 534). However, no balloon or airship has yet been designed for all-purpose optical astronomy.



Fesen envisages a two-balloon catamaran some 120 m long and 15 to 25 m wide, flying over open ocean near Earth's equator. From that vantage point, the telescope could scan both northern and southern skies. It would be safer, too, Fesen says. Hurricanes and cyclones never cross the equator, and upward stratospheric lightning discharges from violent storms ("sprites") rarely occur over open ocean.

High altitude does have its perils, Fesen acknowledges. Harsh ultraviolet radiation from the sun would inevitably take a toll on the airship's fabric. Leaks would also limit the craft's operations, says I. Steve Smith Jr., a former head of NASA's balloon program now at Southwest Research Institute in San Antonio, Texas. Smith estimates that Fesen's proposed airship could stay aloft conducting nearly continuous observations for 3 to 6 months at a stretch before returning to its hangar for 1 to 2 months of repair and restoration.

To observe for weeks or months on end requires the craft to stay in place at a given latitude and longitude. Such "station-keeping" can best be done at approximately 21 km, the altitude at which wind speeds tend to be lowest. "Sixty-five to 70,000 feet [20 to 21 km] is what we tend to think of as the sweet spot," Smith says.

In December, the U.S. Missile Defense Agency awarded Lockheed-Martin $149 million to develop crewless stratospheric airships by 2010 that station-keep at "sweet spot" altitudes over select locations around the country. "At mid-latitudes across the United States, the winds can get pretty ferocious seasonally, well over 120 miles per hour [190 km/h]," Fesen says. "If the military can do that, astronomy [at the equator] is a piece of cake."

To return sharp images, the telescope would also need to compensate for the craft's inevitable jiggling. Part of this problem has already been solved, Fesen says, pointing to the 1-m SUNRISE solar telescope that is scheduled to take its first scientific flight next year. A paper on this balloon-borne telescope's optics (published in the *Proceedings of the International Society for Optical Engineering* in 2004) describes its pointing system, one that is designed to stabilize the target image down to the resolution of the mirror at a speed of up to 30 adjustments per second.

Fesen says telescopes on his proposed craft should start small, at least during any development phase. The scientific payload, including small telescope and pointing hardware, would occupy a desk-sized footprint and weigh up to 15 kg, Fesen says. He estimates that ultimately a 1-meter telescope payload would weigh 75 kg.

"As more capability is brought online, like station-keeping, I think there's a lot of creative people out there, and they're going to find ways to capitalize on that," Smith says. "If you build it, they will come. That's usually been the case in ballooning."

–MARK ANDERSON

Mark Anderson is a writer in Northampton, Massachusetts.

CREDITS (TOP TO BOTTOM): 21ST CENTURY AIRSHIPS INC.; SOUTHWEST RESEARCH INSTITUTE

EDITED BY YUDHIJIT BHATTACHARJEE

On Campus

CROSSED WIRES. While running a mass spectrometer this summer, graduate students Dalila Fondren and Jason McClain of the University of Georgia, Athens, noticed an enormous amount of noise in the output. "We couldn't even see any kind of mass peaks—it was right off the charts," says McClain. After puzzling for a bit, they discovered that the noise was the same frequency as the signal transmitted by the student-run radio station on campus.

While they looked for a fix, McClain (right), Fondren (middle), and their adviser, chemist Nigel Adams (left), worked out a deal with managers of the radio station to keep the research going: The station would go off the air from 7 a.m. to 4 p.m. during weekdays. "Since it's typically slow in the summer, they didn't mind," says Adams, who studies reactions in low-pressure gases. "It was a very amicable arrangement."

Last month, 6 weeks after the noise was detected, a permanent solution was found. Based on a suggestion by an engineer at the radio station, the researchers connected a so-called notch filter to the spectrometer to block the offending frequency. It's unclear what caused the sudden interference, given that the radio station and Adams's lab had coexisted peacefully for years. But ongoing campus construction could have deflected the radio signal into the lab, Adams says.

POLITICS

FREE AGAIN. Political geographer Ghazi Falah, 53, of University of Akron, Ohio, returned home last week after a 23-day detention by the Israeli government. A dual Israeli-Canadian citizen, Falah was taking pictures near the Israel-Lebanon border for his research when he was arrested on suspicions of espionage, 4 days before the current war erupted.

The Israeli government would not explain the arrest or subsequent release. But Falah says his detention was retribution for his academic work, which is critical of Israeli land policies that marginalize Arabs. "There were five interrogators. … Sometimes they tied my hands behind my back," Falah told the Associated Press.

Falah's son Naail is grateful for the support of thousands who petitioned the Israeli judge who eventually ordered his father released. Among the academics who wrote the judge, he noted, were Israeli researchers "who don't agree with my father."

MOVERS

DOCTOR'S DEPARTURE. Richard Carmona, the 17th U.S. Surgeon General, quietly stepped down last month at the end of his 4-year term. A former trauma surgeon from Arizona, the 56-year-old Carmona was less visible than some of his predecessors, focusing on disaster preparedness, childhood obesity, and health disparities. But he made waves this summer with a report on the health risks of secondhand tobacco smoke.

INSIDE GOVERNMENT

DEREGULATOR? Environmentalists cringed last week when the Bush Administration named economist Susan Dudley to head the White House's regulatory affairs office, which reviews federal regulations at agencies such as the Environmental Protection Agency (EPA). Dudley, 51, has directed regulatory studies at the Mercatus Center, a conservative think tank at George Mason University in Fairfax, Virginia, since 2003.

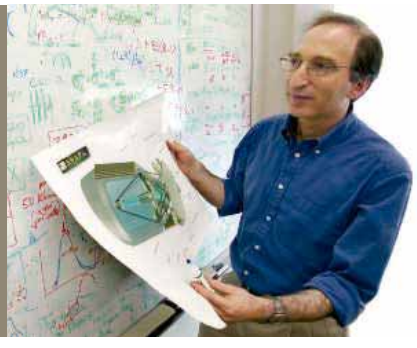
"Inevitably, Dudley sides with some special interest that doesn't want regulating," says Frank O'Donnell of Clean Air Watch in Washington, D.C.

He notes that Dudley advocated against EPA setting tougher standards for smog. (In her comments to EPA, Dudley recommended "nonregulatory approaches" such as public health advisories to address the problem.) A spokesperson for Dudley said she did not want to be interviewed while her nomination was pending.

Dudley would replace John Graham, who left last year for the Pardee RAND Graduate School in Santa Monica, California (*Science*, 28 October 2005, p. 617). O'Donnell says he expects the president to appoint Dudley later this month, while Congress is in recess, "because of the furor that will emerge over her appointment."

Awards >>

ROLLING IN RICHES. What a summer it has been for Saul Perlmutter. In June, the 47-year-old astrophysicist at Lawrence Berkeley National Laboratory in California won a third of the $1 million Shaw Prize in Astronomy (*Science*, 30 June, p. 1871). And last month, he netted another cool $315,000 as winner of the International Antonio Feltrinelli Prize from Italy's Lincei Academy. Both awards recognize Perlmutter's role in discovering that the universe is expanding more and more rapidly over time.



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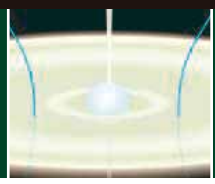
CREDITS (TOP TO BOTTOM): NIGEL ADAMS; DAVID DEAL; FALAH FAMILY HANDOUT VIA AP; ROY KALTSCHMIDT/LAWRENCE BERKELEY NATIONAL LABORATORY



LETTERS

edited by Etta Kavanagh

The Ethics of Influenza Vaccination

IN THEIR POLICY FORUM "WHO SHOULD GET INFLUENZA VACCINE WHEN NOT ALL CAN?" (12 MAY, p. 854), E. J. Emanuel and A. Wertheimer offer valuable contributions to a national discussion on vaccine rationing strategies that could take on tremendous importance if the present concerns of an avian flu pandemic are realized in the United States. I agree that the save-the-most-lives principle of vaccinating those predicted with the highest risk of hospitalization and dying is not appropriate for a pandemic and that the life-cycle allocation principle, which essentially prioritizes youth, is a better approach.

However, I disagree with their investment refinement of excluding children under 13 years old from the highest priority group of 13- to 40-year-olds. Children should be in the top-priority group, since limited vaccine supply would only reach this group. Furthermore, a footnote in their priority table even suggests allocating no vaccine for those under 13 if they could be effectively isolated, an unrealistic and unacceptable proposition. Schoolchildren are considered an important source of community-wide disease transmission, and vaccinating them can reduce mortality in other groups (*1*). The highest relative risk of death in New York City during the 1918 influenza pandemic was for 5- to 14-year-olds (*2*). The investment refinement, based on the amount a person has invested in their lives together with that left to live, fails to consider parental investment; parents invest heavily—both emotionally and economically—in their children. Since many parents are in the highest priority group, I would expect that many would give their share of a vaccine to their children, if they could, before taking it themselves.

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THE POLICY FORUM "WHO SHOULD GET INFLUENZA vaccine when not all can?" by E. J. Emanuel and A. Wertheimer (12 May, p. 854) proposes a "life-cycle principle," by which potential vaccine recipients are prioritized according to their years of expected life, the intent being to save the most years of life over the entire population.

The implementation presented to achieve this end is defective in that it fails to consider possible differential mortality rates with age. If older people are more likely to die from the disease than younger people, more years of life may be saved by giving priority to their treatment.

For example, if healthy 60-year-olds with life expectancies of 19 years have expected mortalities from the disease of 10%, treating them with a universally effective vaccine would save 1.9 years of life on average. If healthy 80-year-olds with 7-year life expectancies had expected mortalities of 50%, treatment would save an average of 3.5 years of life. Thus, more years of life would be saved by treating the older cohort.

Arbitrary age cut-offs, such as those recommended by Emanuel and Wertheimer, should not be promulgated, but priorities should be determined based on the best information available, including relative age-based mortalities.

HARVEY S. FREY

Health Administration Responsibility Project, Inc., 552 12th Street, Santa Monica, CA 90402–2908, USA.

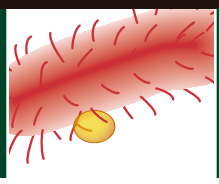
IN THEIR POLICY FORUM "WHO SHOULD GET influenza vaccine when not all can?" (12 May, p. 854), E. J. Emanuel and A. Wertheimer propose that strategies for allocation of influenza vaccine should maximize "the amount a person invested in his or her life balanced by the amount left to live," which is highest in young adults, 13 to 40 years old. They then incorrectly suggest that this "ethical framework" leads to prioritization in vaccinating these young adults over other age groups.

A vaccination program must be based on both projected outcomes of candidate interventions and the value of these outcomes. Projected outcomes cannot be derived without the inclusion of risk information, such as transmissibility or disease severity (see table). Evaluating the effectiveness of various interventions also depends on our value system: Often, public health programs aim to minimize mortality or infection incidence. Emanuel and Wertheimer consider how to value outcomes and advocate for a vaccination strategy without considering the outcome of such a strategy.

An example of critical risk information regarding influenza outbreaks is the fact that children are most responsible for initiating and perpetuating epidemics, particularly children 3 to 4 years old (*1*). Epidemiological and simulation studies demonstrate that influenza vaccination targeted at children can dramatically reduce community-wide transmission (*1, 2–6*). Consequently, it may actually be more beneficial for the elderly, young adults, and the community in general to prioritize vaccination of children, because this strategy limits the overall size of an epidemic and provides indirect protection to all other age groups. There are, of course, ethical issues with exposing one group to the risks of vaccination for the benefit of another group, which must also be taken into consideration.

How magma moves

771



Bacterial compound's dual effects

772

Risk information

Risk component	Definition	Example fact
Risk severity	Severity of consequences of influenza	Influenza mortality is greater for the elderly (*7*).
Risk likelihood	Likelihood of contracting influenza if unvaccinated	The elderly are slightly less likely to contract influenza than are young adults (*8*).
Transmissibility	The likelihood that one infected person will infect another unvaccinated person	Young children are responsible for most transmission (*1*,*9*).
Vaccine effectiveness	The extent to which vaccination decreases the risk likelihood and severity, and prevents virus transmission	Influenza vaccine is less effective for the elderly than for young adults (*10*).

Available criteria by which to judge health policies are somewhat lacking, and we applaud Emanuel and Wertheimer for proposing a new ethical framework to address this deficit. However, neglecting important epidemiological details can lead to inappropriate strategies that fail to achieve the ethical objective. Protecting young adults may in fact be best accomplished by not vaccinating them directly, but by vaccinating those responsible for the majority of transmission.

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Response

DECIDING WHO SHOULD TAKE PRIORITY FOR scarce influenza vaccine in a pandemic entails decisions about minimizing bad outcomes. The issue is what ethical justification can be given for who will get the vaccine. We had two main objectives: (i) to challenge the National Vaccine Advisory Committee (NVAC) and Advisory Committee on Immunization Policy (ACIP) recommendation that is based on the utilitarian "save-the-most-lives" approach, and (ii) to argue that the investment modification of the life-cycle principle is the least bad solution to a tragic situation.

Silverstein agrees with us regarding (i), but rejects (ii). In evaluating a life, we look at future possibilities and also at what has already been invested in the life by the person and by others. A 20-year-old has had great investment that is largely unfulfilled, while a 2-year-old has had minimal investment and a 65-year-old has had great investment that is largely fulfilled. But even if we were to abandon the investment modification of the life-cycle principle, we would argue that the life-cycle principle is superior to the save-the-most-lives principle.

The Letter by Galvani *et al.* makes us believe we were not sufficiently clear that we reject the traditional public health approach to "minimize mortality or infectious incidence." No one does—or should—just count numbers of dead bodies to determine which course of action is better. That approach is morally simplistic. It fails to account for our assessments about premature deaths and is ethically indefensible.

We do agree with Galvani *et al.* that the NVAC and ACIP approach is inadequate on both ethical and practical accounts. From the

Excess mortality rates in 1918–19 pandemic

Age cohort	Excess mortality due to influenza 1918–19 (per 10,000 population)
<5	72
5–14	19
15–24	58
25–44	76
45–64	21
>64	15
All ages	53

ethical perspective, we delineate the investment modification of the life-cycle principle. Although we agree that vaccinating the young rather than the old is probably the better strategy to minimize spread of influenza, such spread among young children can be handled by social isolation—closing schools and other congregating venues. However, we argued that the investment refinement of the life-cycle principle combined with the public-order principle "should be the ultimate objective of all pandemic response measures." This means if the best way to save the lives of those 13 to 40 is to vaccinate children 4 and 5 years of age because social isolation won't work, then that should be the policy.

In considering life years saved, we should be careful to use realistic numbers, not exaggerated hypotheticals of 50% mortality proposed by Frey. In the 1918–19 pandemic, the excess mortality rates by age group are shown in the table (*1*).

These data indicate that if we treated 15- to 24-year-olds with a universally effective vaccine, we would save on average 60 years per person who might have died or 0.348 years per person in the age cohort. For the over-64-year-olds, it would be just 0.023 years per person in the age cohort—15-fold less. And this assumes the vaccine is equally effective in young as in old people, yet a recent review of 31 studies shows that the "adjusted odds ratio of responses in elderly versus young adults [ranges] from 0.24 to 0.59 in terms of seroconversion and seroprotection" to influenza vaccine (*2*).

We argued that our life-cycle approach was not equivalent to saving the most life years. There is a good ethical argument that even if vaccinating the elderly saved the most life years, one should prefer the young over the old because the young have more unfulfilled life.

EZEKIEL J. EMANUEL AND ALAN WERTHEIMER

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Complex Choices Better Made Unconsciously?

IN THEIR REPORT "ON MAKING THE RIGHT choice: The deliberation-without-attention effect" (17 Feb., p. 1005), A. Dijksterhuis and colleagues reported the intriguing finding that when participants had to choose among four cars on the basis of various attributes, a period of conscious reflection worsened performance. They took this as evidence that complex choices are better when made unconsciously. A close examination of their methods, however, suggests a less startling interpretation.

Because of the easily confusable statements about the four cars, the 4-min period of reflection would cause considerable memory interference and leave participants utterly confused (was it the Hatsdun that had good handling and the Kaiwa no cupholders, or the other way round?). Memory research in the Bartlettian tradition has revealed many examples of such self-generated interference (*1*). The unconscious group made their decision after a similar 4-min period filled with a distractor task. Knowing that they would have no further opportunity for reflection prior to being required to make their choice, these individuals probably just made their decision at the end of the study period based on their overall impression of which car was best. This alternative account makes a simple and testable prediction, namely, that memory recall will be worse in the conscious condition.

An interesting but unnoted aspect of the findings was that the deliberation group chose the best car on only about 25% of occasions, exactly at chance. Does conscious deliberation yield no more than random results? The alternative account suggested here offers an explanation: It must have been because these individuals were faced with an insurmountable memory challenge and were completely confused about which attributes went with which car.

In any event, the decision problem presented in this study is very unlike the way we normally deliberate about a problem. When choosing between cars, we don't expend effort struggling to recall their attributes; we familiarize ourselves with the relevant attributes during the information search stage, and if we can't recall some attribute, we find it out. Dijksterhuis *et al.*'s findings would be altogether more compelling if they were replicated in a situation in which the 4-min deliberation period was spent studying the cars' attributes. But the likelihood is that under such circumstances, the best alternative would be selected by close to 100% of participants.

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Reference

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Response

IN OUR WORK ON THE "DELIBERATION-WITH-out-attention" effect, we found that, under complex decision circumstances, unconscious thinkers made better decisions than conscious deliberators. Conscious deliberators suffer from the low memory capacity of consciousness, which renders it impossible for them to take into account substantial amounts of information simultaneously. Unconscious thinkers, on the other hand, are not negatively affected by such capacity constraints. Shanks offers alternative explanations for our findings for both conscious deliberators and unconscious thinkers.

Shanks argues that our conscious thinkers may have faced memory problems. However, memory problems are not causing the effects we see. We have shown that even when the statements are presented in blocks (i.e., first all information on car A, then on car B, etc.), conscious deliberation still produces poor results (*1*). In addition, we have shown that even when people do have all the information at hand during conscious deliberation, it still produces poor results (*2*).

Shanks's suggestion that unconscious thinkers simply stick to the initial decision they made immediately after processing the information is not correct. In other experiments (*1–3*), we have compared unconscious thinkers with people who made decisions immediately after having received all the information, and unconscious thinkers performed better. Unconscious thought does lead to changes in preference, and it does so for the better.

Shanks also notes that under complex conditions, decisions made by conscious deliberators are no better than chance. Although conscious deliberation itself cannot be said to be random, the decisions produced by conscious deliberation are under some circumstances not superior to randomly generated decisions. There are moderators at work here, of course (e.g., expertise). Thus, the idea that conscious deliberation before making decisions is always good is simply one of those illusions consciousness creates for us.

Finally, Shanks observed that our experiments do not reflect the way people normally make decisions. This is true, as is usually the case with lab experiments. However, that is exactly the reason we included two field studies in our Report. In the field studies, people made real decisions with real consequences. These studies also confirmed the "deliberation-without-attention" hypothesis.

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CORRECTIONS AND CLARIFICATIONS

News Focus: "Social animals prove their smarts" by E. Pennisi (23 June, p. 1734). On page 1738, Mark Bekoff's name was spelled incorrectly.

News Focus: "At home on a no-frills tell" by A. Lawler (9 June, p. 1460). Archaeologist Joan Oates was incorrectly described as having worked in what is now Israel. Oates's teacher, Dorothy Garrod of Cambridge University, did work there in the 1930s, but Oates was not part of the excavation.

News of the the Week: "PTO wants to tap experts to help patent examiners" by E. Kintisch (19 May, p. 982). The affiliation of Beth Simone Noveck was incorrectly reported. She is a professor at Manhattan's New York Law School, not New York University Law School.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Gene Regulatory Networks and the Evolution of Animal Body Plans"

Jerry A. Coyne

Davidson and Erwin (Reviews, 10 February 2006, p. 796) argued that known microevolutionary processes cannot explain the evolution of large differences in development that characterize phyla. Instead, they proposed that phyla arise from novel evolutionary processes involving large mutations acting on conserved core pathways of development. I question some of their assumptions and show that natural selection adequately explains the origin of new phyla.

Full text at www.sciencemag.org/cgi/content/full/313/5788/761b

RESPONSE TO COMMENT ON "Gene Regulatory Networks and the Evolution of Animal Body Plans"

Douglas H. Erwin and Eric H. Davidson

Contrary to Coyne's assertions, our paper did not advocate a macromutational innovation of phyla but considered the consequences of the introduction of developmental constraints for the evolution of gene regulatory networks based on recent empirical studies of gene regulatory networks.

Full text at www.sciencemag.org/cgi/content/full/313/5788/761c

BIOMEDICINE

A Cellular and Molecular Foundation for Understanding Cancer

George Klein

Around the middle of the last century, the image of cancer metamorphosed from an enigmatic disease to the multistep clonal-subclonal evolution of cell populations toward increased independence of growth control, a Darwinian process. The changes in cell phenotype (and, as they became known, in the corresponding genetic determinants) that accompanied cancer turned out to be relevant for understanding normal growth and development. Until then, cancer biology had been the junior partner or poor relative of cell biology. During the next two decades, their positions were reversed and cancer biology took center stage. The 1970s saw a panvirological interregnum, when most if not all tumors were attributed to manifest or cryptic viruses. But then the discovery of oncogenes and tumor suppressor genes, heralded by the chase for tumor viruses, displaced viruses from the limelight. While many virologists departed from the cancer field, an ever-increasing number of cell biologists entered. Robert Weinberg was among the most important pioneers in this reorienting of research. His pivotal discovery of the first oncogene mutation (ras) in a human tumor put an end to the notion that oncogenes could only be activated by viruses. It was one of the main starting points for the molecular analysis of the development and progression of tumors. Weinberg's work on the cooperativity between different oncogenes was another milestone. Now, in *The Biology of Cancer*, Weinberg offers students and researchers alike a comprehensive view of the field.

The book, unlike some by authors who have made far fewer original contributions, does not emphasize Weinberg's substantial role in the development of cancer biology. Like a medieval monk, he chose to hide his contributions within a nearly complete coverage of the field. It therefore seems appropriate to bring them up, if only to make the point that Weinberg's incessant publication of original research would appear incompatible with the production of this amazing, singly authored book

Weinberg, who is widely admired as a teacher, has taken great pains to be simultaneously comprehensible for the student and interesting for the expert. The book offers an abundance of didactic, and sometimes masterful, illustrations. Each chapter is followed by a list of key concepts, prospects, and "thought questions," designed to stimulate the active involvement of the reader. A CD-ROM included with the book contains all of the illustrations in PowerPoint format along with additional sidebars, mini-lectures, and movies. An accompanying poster summarizes key signaling pathways involved in tumor genesis and development in humans. The set offers a veritable gold mine for lecturers and students.

The Biology of Cancer
by Robert A. Weinberg
Garland Science, New York, 2006. 864 pp + CD-ROM. $140, £89.99. ISBN 0-8153-4078-8. Paper, $99, £41.99. ISBN 0-8153-4076-1.

The book covers an astounding breadth of material. Both descriptive and analytical, it amalgamates the historical background and modern developments. The text integrates cancer biology with gross and histopathology. If not solidly linked, laboratory research and epidemiology are at least juxtaposed. Weinberg gives detailed consideration to existing and prospective therapeutic approaches. He provides convincing examples of the value of high-throughput molecular approaches in diagnostic subclassification and prognostication. His analysis of the potential for a rational targeting, heralded by drugs such as Gleevec and Herceptin, includes explanations of when these and similar approaches are frustrated and offers clear guidelines for further progress. The author blends analytical detail with conceptual projections. The lucid text carries the reader forward at a steady pace, and there is never a boring moment.

One must look hard to find the book's shortcomings. In areas outside his direct experience, Weinberg occasionally favors hypotheses that have already been disproved. In contrast to Epstein-Barr virus–driven immunoblastomas, the development of Burkitt's lymphoma does not require immunosuppression. Epstein-Barr virus does not express its mitogenic program in Burkitt's lymphoma or nasopharyngeal carcinoma cells and does not drive their proliferation. The immunoglobulin gene–*MYC* translocations are accidents of normal immunoglobulin gene rearrangements or class switching. They occur in cancer-free people as well and do not require malaria-induced proliferations. Nor is the tumorigenic phenotype dominant in hybrids between virus-induced tumors and normal cells. (The recessiveness of tumorigenicity in tumor-normal hybrids also applies to most virus-induced tumors, with the exception of some mouse lymphoma-lymphocyte hybrids.) But it would be petty to belabor minor points like these.

Cancer colonies. Liver metastases (white) often arise in patients with advanced colon cancer, after cancer cells migrate through the portal vein.

A more important critique may be directed against the relatively low profile Weinberg accords cancer epigenetics. As he and most cancer geneticists readily acknowledge, epigenetics has now emerged as a worthy partner of cancer genetics. But it is still often mentioned in parentheses—(methylation)—rather than on equal footing. In addition, there is more to epigenetics than changes in DNA methylation. Chromatin structure and the stringency of parental imprinting are emerging as important variables that can influence the likelihood of tumor development.

Another new and rapidly growing field, concerning the effect of the microenvironment on initiated precancerous or even fully fledged cancer cells, is discussed in detail. But some of the most remarkable cancer-related effects receive less attention than they deserve. For example, there are the "normalization" of tumor cells by contact with normal cells and the suppression of tumorigenicity by recreating three-dimensional tissue structure. That approach, pioneered

CREDIT: FIG. 2.2B FROM *THE BIOLOGY OF CANCER*/COURTESY PETER ISAACSON

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by Mina Bissell, shows that "phenotype can override genotype" (*1*), irrespective of the number of genetic changes in the tumor cells. The book does not even mention the most spectacular case, Beatrice Mintz's demonstration that highly malignant mouse teratoma cells can, if placed in an early embryonic environment, be induced to develop all normal tissues of the mouse (*2*). On the other hand, Weinberg provides a very interesting discussion on epithelial-mesenchymal transition during the development of invasive tumors and the reverse, mesenchymal-epithelial transition in late tumor progression. These transitions, which mimic certain stages of embryonic development, are very relevant for an understanding of interactions between cells and their normal or modified neighbors.

The space and detail Weinberg devotes to general and tumor immunology are somewhat surprising in view of his repeated emphasis of the "state of flux" of that particular field. We still lack a decisive answer to the original question: Does the immune system regard tumor cells as self or as nonself? Most of the observed nonself responses with an indisputable rejection potential have involved virus-transformed cells. The power of such responses can be demonstrated by the ability of immunocompetent T cells to bring even widely disseminated Epstein-Barr virus–driven immunoblastomas in immunodeficient patients to complete regression. Most nonviral tumors never have to face a comparable recognition. Although antibodies are (as the book shows) widely detected against many tumor proteins, this may be the symptom of a response rather than evidence of rejection-mediating effectors. Many ongoing efforts to mobilize tumor inhibitory immune responses may be akin to breaking tolerance to self. This approach is well presented in the book, but the question remains how far tumor inhibitory immune responses can be driven in the face of multifactorial protection against autoimmune reactions. Weinberg does not hesitate to reveal his own ambivalence, while doing justice to the current efforts that dominate the field.

The Biology of Cancer is no doubt the definitive statement on its topic today. But nothing remains definitive for too long in this field. An updated edition will be needed in a few years' time. By then, the RNA revolution and particularly the role of the regulatory microRNAs that can play both oncogene and tumor suppressor roles (*3*) will have delivered a vast body of new information. The concept of junk DNA may have been abandoned altogether. But however revolutionary these developments may be, they will stand on the solid foundation compiled in Weinberg's monumental book.

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10.1126/science.1131416

HISTORY OF SCIENCE

On Smell and Scientific Practice

Miriam Solomon

A delightful book about the science of smell, *The Secret of Scent* takes the reader through a tour of the almost infinite range of human olfactory possibilities. Luca Turin also presents the recent history of theories of smell, culminating with his own frequency theory. Turin possesses an unusually sensitive nose and has the ability to detect and describe, like a wine expert, the character of individual odors and complex scents, natural and synthetic, pleasing and noxious. A perfume guide he wrote (*1*) became a best seller in France. His perfume reviews (*2*) contain such colorful lines as "This thing smells like an infant's breath mixed with his mother's hair spray.… What Rush can do, as all great art does, is create a yearning, then fill it with false memories of an invented past" and "Python … belongs in a tree shaped diffuser dangling from the rearview mirror of a Moscow taxi." The success of his perfume guide led to invitations to visit and consult with scent and perfume manufacturers, from which Turin learned much about the process of creation of scent.

In part because of this unusual access to perfumery materials and manufacture, Turin has found the leading theory of smell—that humans detect small volatile molecules by assessing the shape of the molecule or part of the molecule—unsatisfactory. Shape theories were originally proposed by Linus Pauling (*3*) and R. W. Moncrieff (*4*) in the 1940s and subsequently developed by John Amoore and others. Turin observes that research on creating new smell molecules is trial and error. Data mining for correlations between molecular shape and smell has not generated useful predictions. Scent manufacturers typically synthesize 1000 new molecules to get one that they can use. Turin observes that, contrary to the predictions of shape theories, molecules very different in shape can sometimes smell the same (e.g., boranes smell sulfurous) and molecules very similar in shape can smell different (e.g., isotopes of the same molecule such as acetophenone and deuterated acetophenone).

Turin has a Ph.D. in biophysics. At the time that he developed his theory of smell, he was a lecturer at University College

The Secret of Scent
Adventures in Perfume and the Science of Smell

by Luca Turin

Faber and Faber, London, 2006. 217 pp. £12.99. ISBN 0-571-21537-8. Forthcoming from Ecco, New York. ISBN 0-06-113383-3.

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BROWSING

Strategies of Commitment and Other Essays. Thomas C. Schelling. Harvard University Press, Cambridge, MA, 2006. 355 pp. $39.95, £25.95, €36.90. ISBN 0-674-01929-6.
Schelling shared the 2005 Nobel Prize in Economic Sciences for use of game theory to understand the bases of conflict and cooperation. In this collection of previously published articles and excerpts, he offers informative perspectives on a wide range of topics. Some, such as the nature of commitment and the avoidance of nuclear war, have interested Schelling since early in his career. (A 1960 book review of his helped inspire *Dr. Strangelove.*) Others reflect more recent concerns, including end-of-life controversies, addictions, global warming, and using prices as regulatory instruments to protect the environment. One chapter reprints his 1971 "Dynamic Models of Segregation," which demonstrated a tipping point in the racial composition of neighborhoods. Several of the essays consider cases in which the usual assumptions of economists (e.g., rational decision-makers) do not hold. Anyone interested in the behaviors of individuals or societies will find many of the pieces thought-provoking; in one, Schelling even argues "that there are free lunches all over just waiting to be discovered or created."

London. His research has ranged from electrophysiology to protein semiconductors and work on solitons. He has always read widely in the fields related to his research. He also has a taste (unusual among researchers) for used science books, which he purchases on his travels and which connect him with both recent history of science and non-Anglophone science.

The author knew of the theories of Malcolm Dyson (*5*) and Robert Wright (*6*), which claimed that smell (like sound and color perception) is based on frequency detection. For smell, the frequencies detected and measured are the vibrational frequencies of odorant molecules. Historically, the frequency theory faltered on the observation that enantiomers (mirror images of the same molecule, having the same vibrational spectrum) sometimes smell different and on the lack of a known mechanism for measuring vibrational frequency of molecules. Turin noted, however, that (as mentioned above) shape theories also have substantial contrary observations. He argues for a balanced look at all the evidence, and he considers the ability of each theory to accommodate contrary observations. Building on his earlier work on the electrical conductivity of proteins, Turin proposes that smell receptors are sensitive to particular ranges of vibrational frequency of molecules and use electron tunneling to transmit an electric signal when the appropriate odorant molecule is in the receptor. (This explanation is an interesting application of quantum mechanics to understand a physiological phenomenon.) Genomic sequencing by Linda Buck and her colleagues has identified about 350 different smell receptors in humans (*7*, *8*). Turin does not suggest that each smell receptor responds to a different range of frequency. He thinks it more likely that classes of smell receptors respond to the same ranges of frequency but fit different sizes and shapes of molecule. (In this way, Turin explains the findings about enantiomers, but also complicates his theory with a shape component governing the affinity of odorants for receptors.)

Journalist Chandler Burr's widely read and (mostly) favorably reviewed book (*9*) has already told the story of the development and reception of Turin's theory. Academic and commercial smell researchers alike have been largely dismissive of Turin's hypothesis. Turin submitted a paper proposing his spectroscopic mechanism for olfactory reception to *Nature*, where it was rejected after a lengthy review process (described in Burr's book). The paper was then published in a specialty journal, *Chemical Senses* (10), and Turin subsequently presented a refined version of his theory (*11*). Skepticism about Turin's theory has been evident in *Nature Neuroscience*, which published a scathing review of Burr's book (*12*), a short paper reporting three experiments that failed to support the vibration theory (*13*), and an editorial commenting on that paper and complaining about "the extraordinary—and inappropriate—degree of publicity that the theory has received from uncritical journalists" (*14*).

Sensing scents. M. Maumus's advertising poster for the perfume Nelombo (1932).

Burr saw in the early responses to Turin's theory a "failure of the scientific process," but he has been accused of excessive partiality toward his subject. *The Secret of Scent* is an interesting sequel, and partial corrective, to Burr's account. It is much more a book about science than about scientists, and it is refreshingly non-egotistical. Turin does not describe his own theory until page 160, and he presents the relevant contributions of many scientists from a range of scientific subdisciplines, including organic chemistry, the physics of electron tunneling, and the physiology of insect olfaction. Of particular note is Turin's coverage of findings from Soviet and Russian researchers.

Intended for a general audience, *The Secret of Scent* skillfully presents the necessary concepts from physics and chemistry. For example, Turin explains molecular vibrations by using an analogy with dance: vibrations can be local to parts of the molecule (like head movements in Indian dance) or involve the whole molecule (like 1970s disco). The book is not a polemic, but rather a straightforward presentation of odor, theories of odor, and the author's theory of odor in particular.

Turin continues to work with his theory, presently in a corporate rather than an academic context. He is currently the chief scientist of Flexitral, a privately held U.S. company that uses his theory to design new scents, seeking molecules that are cheap to synthesize and have favorable toxicological and environmental profiles. Turin claims a success rate of 10% (one in ten syntheses produces a commercially viable molecule), which is two orders of magnitude above the industry average. Perhaps he will persuade the corporate world to take his frequency theory seriously before the academic community does.

As one would expect, Turin wishes his theory had found a more positive reception. Insofar as he assigns blame for its current fate, he faults the process of peer review. Turin believes that in areas requiring a high degree of specialized knowledge, any competent referee will have a conflict of interest. Competition will get in the way of a fair review. Moreover, he thinks that interdisciplinary research is especially vulnerable to deficient review, because it is difficult to find reviewers with the required broad range of expertises.

The Secret of Scent should appeal to anyone curious about smell, whether as a researcher or an intrigued layperson. It also touches on various aspects of science practice and policy, including scientific creativity, the difficulties of interdisciplinary research, the importance of unusual skills, and the consequences of unusual access to data. And Turin's story will also attract those, like myself, interested in scientific controversy.

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SCIENCE COMMUNICATION

Public Acceptance of Evolution

The acceptance of evolution is lower in the United States than in Japan or Europe, largely because of widespread fundamentalism and the politicization of science in the United States.

Jon D. Miller,[1]* Eugenie C. Scott,[2] Shinji Okamoto[3]

The concept of the evolution of humans from earlier forms of life is unacceptable to biblical literalists and causes concern even among some holders of less conservative religious views. Catholics and mainstream Protestants generally accept variations of a theological view known as theistic evolution, which views evolution as the means by which God brought about humans, as well as other organisms. Evolution is nonetheless problematic to some of these nonliteralist Christians, because it implies a more distant or less personal God (*1*–*3*). Efforts to insert "intelligent design" into school science curricula seek to retain the divine design of humans while remaining agnostic on earlier creationist beliefs in a young Earth and the coexistence of humans and dinosaurs (*2*, *4*).

Beginning in 1985, national samples of U.S. adults have been asked whether the statement, "Human beings, as we know them, developed from earlier species of animals," is true or false, or whether the respondent is not sure or does not know. We compared the results of these surveys with survey data from nine European countries in 2002, surveys in 32 European countries in 2005, and a national survey in Japan in 2001 (*5*). Over the past 20 years, the percentage of U.S. adults accepting the idea of evolution has declined from 45% to 40% and the percentage of adults overtly rejecting evolution declined from 48% to 39%. The percentage of adults who were not sure about evolution increased from 7% in 1985 to 21% in 2005. After 20 years of public debate, the public appears to be divided evenly in terms of accepting or rejecting evolution, with about one in five adults still undecided or unaware of the issue. This pattern is consistent with a number of sporadic national newspaper surveys reported in recent years (*6*–*10*).

A dichotomous true-false question format tends to exaggerate the strength of both positions. In 1993 and 2003, national samples of American adults were asked about the same statement but were offered the choice of saying that the statement was "definitely true, probably true, probably false, definitely false," or that they did not know or were uncertain. About a third of American adults firmly rejected evolution, and only 14% of adults thought that evolution is "definitely true." Treating the "probably" and "not sure" categories as varying degrees of uncertainty, ~55% of American adults have held a tentative view about evolution for the last decade.

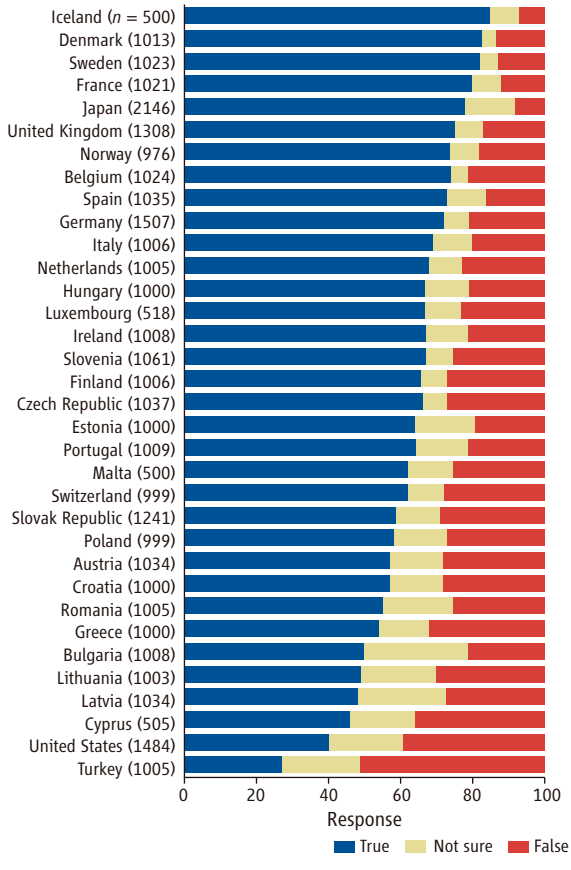
This pattern is different from that seen in Europe and Japan. Looking first at the simpler true-false question, our analysis found that significantly (at the 0.01 to 0.05 level by difference of proportions) (*11*) more adults in Japan and 32 European countries accepted the concept of evolution than did American adults (see figure, right). Only Turkish adults were less likely to accept the concept of evolution than American adults. In Iceland, Denmark, Sweden, and France, 80% or more of adults accepted the concept of evolution, as did 78% of Japanese adults.

A cross-national study of the United States and nine European nations in 2002–2003 used the expanded version of the question. The results confirm that a significantly lower proportion of American adults believe that evolution is absolutely true than adults in nine European countries [see fig. S1 in the Supporting Online Material (SOM)]. A third of American adults indicated that evolution is "absolutely false"; the proportion of European adults who thought that evolution was absolutely false ranged from 7% in Denmark, France, and Great Britain to 15% in the Netherlands.

Regardless of the form of the question, one in three American adults firmly rejects the concept of evolution, a significantly higher proportion than found in any western European country. How can we account for this pattern of American reservations about the concept of evolution in the context of broad acceptance in Europe and Japan?

First, the structure and beliefs of American fundamentalism historically differ from those of mainstream Protestantism in both the United States and Europe. The biblical literalist focus of fundamentalism in the United States sees Genesis as a true and accurate account of the creation of human life that supersedes any scientific finding or interpretation. In contrast, mainstream Protestant faiths in Europe (and their U.S. counterparts) have viewed Genesis as metaphorical and—like the Catholic Church—have not seen a major contradiction between their faith and the work of Darwin and other scientists.

To test this hypothesis empirically, a two-group structural equation model (SEM) (*12*, *13*) was constructed using data from the United States and nine European countries (see statistical analyses in SOM). The SEM allows an examination of the relation between several variables simultaneously on one or more outcome variables. In this model, 10 independent variables—age, gender, education, genetic literacy, religious belief, attitude toward life, attitude toward science and tech-



Public acceptance of evolution in 34 countries, 2005.

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nology (S&T), belief in S&T, reservations about S&T, and political ideology—were used to predict attitude toward evolution. The total effect of fundamentalist religious beliefs on attitude toward evolution (using a standardized metric) was nearly twice as much in the United States as in the nine European countries (path coefficients of –0.42 and –0.24, respectively), which indicates that individuals who hold a strong belief in a personal God and who pray frequently were significantly less likely to view evolution as probably or definitely true than adults with less conservative religious views.

Second, the evolution issue has been politicized and incorporated into the current partisan division in the United States in a manner never seen in Europe or Japan. In the second half of the 20th century, the conservative wing of the Republican Party has adopted creationism as a part of a platform designed to consolidate their support in southern and Midwestern states—the "red" states. In the 1990s, the state Republican platforms in seven states included explicit demands for the teaching of "creation science" (*1*). There is no major political party in Europe or Japan that uses opposition to evolution as a part of its political platform.

The same SEM model discussed above offers empirical support for this conclusion. In the United States, the abortion issue has been politicized and has become a key wedge issue that differentiates conservatives and liberals. In the SEM, individuals who held strong pro-life beliefs were significantly more likely to reject evolution than individuals with pro-choice views. The total effect of pro-life attitudes on the acceptance of evolution was much greater in the United States than in the nine European countries (–0.31 and –0.09, respectively) [see Statistical Analyses section of Supporting Online Material].

The same model also documents the linkage of religious conservative beliefs and a conservative partisan view in the United States. The path coefficient for the relation between fundamentalist religious views and self-identification as a conservative was 0.26 in the United States and 0.17 in the nine European countries. The path coefficient between pro-life views and self-identification as a conservative was 0.20 in the United States and 0.06 in the nine European countries. Because the two-group SEM computes path coefficients on a common metric, these results are directly comparable and the impact of fundamentalist religious beliefs and pro-life attitudes may be seen as additive (*12*, *13*).

Third, genetic literacy has a moderate positive relationship to the acceptance of evolution in both the United States and the nine European countries. This result indicates that those adults who have acquired some understanding of modern genetics are more likely to hold positive attitudes toward evolution. The total effect of genetic literacy on the acceptance of evolution was similar in the United States and the nine European countries.

Although the mean score on the Index of Genetic Literacy was slightly higher in the United States than the nine European countries combined, results from another 2005 U.S. study show that substantial numbers of American adults are confused about some of the core ideas related to 20th- and 21st-century biology. When presented with a description of natural selection that omits the word evolution, 78% of adults agreed to a description of the evolution of plants and animals (see table S2 in SOM). But, 62% of adults in the same study believed that God created humans as whole persons without any evolutionary development.

It appears that many of these adults have adopted a human exceptionalism perspective. Elements of this perspective can be seen in the way that many adults try to integrate modern genetics into their understanding of life. For example, only a third of American adults agree that more than half of human genes are identical to those of mice and only 38% of adults recognize that humans have more than half of their genes in common with chimpanzees. In other studies (*1*, *14*, *15*), fewer than half of American adults can provide a minimal definition of DNA. Thus, it is not surprising that nearly half of the respondents in 2005 were not sure about the proportion of human genes that overlap with mice or chimpanzees.

These results should be troubling for science educators at all levels. Basic concepts of evolution should be taught in middle school, high school, and college life sciences courses and the growing number of adults who are uncertain about these ideas suggests that current science instruction is not effective. Because of the rapidly emerging nature of biomedical science, most adults will find it necessary to learn about these new concepts through informal learning opportunities (*15*–*17*). The level of adult awareness of genetic concepts (a median score of 4 on a 0-to-10 scale) suggests that many adults are not well informed about these matters. The results of the SEM indicate that genetic literacy is one important component that predicts adult acceptance of evolution.

The politicization of science in the name of religion and political partisanship is not new to the United States, but transformation of traditional geographically and economically based political parties into religiously oriented ideological coalitions marks the beginning of a new era for science policy. The broad public acceptance of the benefits of science and technology in the second half of the 20th century allowed science to develop a nonpartisan identification that largely protected it from overt partisanship. That era appears to have closed.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5789/765/DC1

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IMMUNOLOGY

When F-actin Becomes Too Much of a Good Thing

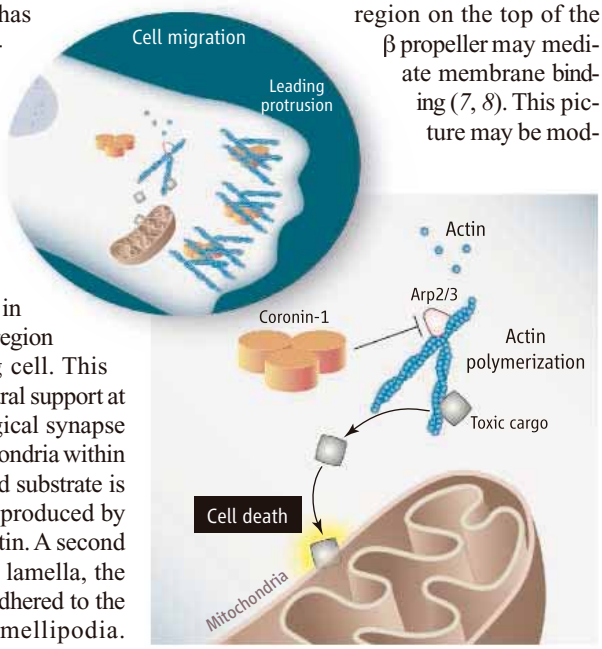
An excess of a protein that regulates actin assembly in white blood cells unexpectedly kills the cell by altering mitochondrial function.

Michael L. Dustin

T lymphocytes are highly mobile and long-lived cells that alternate between circulating in the blood and migrating rapidly in tissue in search of antigens. The actin cytoskeleton, the cell's diverse and dynamic infrastructure, plays a key role in this process, and much attention has been focused on branched networks of filamentous actin (F-actin). These fibrous networks are generated through the activity of the actin-related protein 2/3 (Arp2/3) complex. This complex forms F-actin structures that enable T cells to migrate (see the figure) and to forge a signaling synapse at the interface of an activated T cell and an antigen-presenting cell. Thus, F-actin has been viewed as a "good thing" that is necessary for cell motility and sustained cell signaling. On page 839 of this issue, Föger *et al.* (*1*) suggest that there can be too much of a good thing. Deficiency in the protein coronin-1, a natural antagonist of Arp2/3, increases F-actin content in a T cell, but in so doing it inhibits chemotactic responses and decreases mitochondrial membrane potential, leading to cell death. Surprisingly, loss of coronin-1 has no consequences for immunological synapse formation or T cell activation.

F-actin is a building block of different cytoskeletal structures. Activation of the Arp2/3 complex leads to the formation of branched F-actin filament networks that generate protrusive force and retrograde F-actin flow in the lamellipodium, the extended region at the front end of a migrating cell. This form of F-actin also lends structural support at the periphery of the immunological synapse and moves organelles like mitochondria within the cytoplasm. Arp2/3's preferred substrate is short F-actin filaments that are produced by cofilin, a protein that severs F-actin. A second form of F-actin is found in the lamella, the region of the cell that is firmly adhered to the substrate and that extends lamellipodia. F-actin filaments in the lamella are stabilized by tropomyosin (*2*), a protein that inhibits both cofilin and Arp2/3. Nonmuscle myosin works with these filaments to generate force for cell movement (*3*). A third type of actin network is based on F-actin anchored to the plasma membrane—for example, the spectrin cytoskeleton in a red blood cell (*4*). Although coronin-1 concentrates in dynamic actin protrusions, it inhibits the Arp2/3 complex and may bridge F-actin with membranes. This unique mix of potential actin-regulatory characteristics has led to speculation about cornonin-1 function in lymphocyte homeostasis and activation (*5*, *6*).

Coronin-1 is a homotrimeric cytoplasmic protein with a single amino-terminal β-propeller domain, a linker peptide that appears to be an integral component of the β-propeller domain, and a carboxyl-terminal coiled-coil domain (*7, 8*). The coiled-coil domain inhibits Arp2/3 (*7*). The F-actin binding site appears to be a conserved basic motif on the underside of the β propeller, whereas a less conserved acidic region on the top of the β propeller may mediate membrane binding (*7*, *8*). This picture may be modified as the recently determine structure allows more precise mutation studies.



Does F-actin deliver toxic cargo to mitochondria? In a migrating cell, coronin-1 inhibits Arp2/3 at the leading edge. It may also prevent the delivery of toxic cargo to mitochondira, such as the apoptotic protein Bax.

CREDIT: P. HUE/*SCIENCE*

Foger *et al.* show that coronin-1–deficient T cells develop normally, but migration and survival defects lead to T lymphopenia. The migration defects are consistent with earlier studies linking coronin-1 to processes like motility and phagocytosis in macrophages. The importance of Arp2/3 inhibition by coronin-1 in motility may be understood in terms of the dominant role of less dynamic F-actin that is in complex with tropomyosin in cell motility (*2*). It is possible that coronin-1 may play an important role in the transition from high Arp2/3 activity in the lamellipodium to the more stable actin structures in the lamella. Because coronin-1 bridges F-actin to the membrane, it may also play a role in shaping protrusions. This, in combination with inhibiting Arp2/3 activation, may establish an appropriate cortical actin scaffold in cellular protrusions that allow effective migration in the three-dimensional setting of a lymph node, which differs substantially from the two-dimensional settings often studied in vitro. In three-dimensional settings, motility may depend more on the shape of the protrusions and the use of mechanical anchoring than on adhesion to couple contractile force to movement.

The relation between F-actin and mitochondrial membrane potential has been noted in studies with yeast and mammalian cells. Mutations that lead to increased F-actin accumulation in yeast decrease mitochondrial membrane potential and abrogate growth on carbon sources that require oxidative phosphorylation. Opening of voltage-dependent anion channels (VDACs) is one mechanism that reduces mitochondrial membrane potential. F-actin can modulate VDACs by controlling the pool of gelsolin, an actin-severing protein that binds and closes VDACs (*9*, *10*). However, this effect was not observed with mouse gelsolin, so it likely is not the specific mechanism of action in coronin-1–deficient T cells. However, the general principle that large amounts of F-actin could work by sequestering a soluble binding protein that has a dual role in controlling VDAC and F-actin may apply. This sequestration idea is also supported by the observations of Foger

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et al. and others that simple manipulation of F-actin—including treatment with latrunculin A to decrease F-actin or treatment with jasplakinolide to increase F-actin—either increases or decreases mitochondrial membrane potential, respectively.

Alternatively, F-actin may be critical for delivering proapoptotic molecules to mitochondria (*11*). F-actin has a role in delivery of other "cargo" to mitochondria. For example, dynamin-related protein 1 is delivered by F-actin in order for mitochondria to undergo fission (*12*). In yeasts, the F-actin anchoring complex on mitochondria has been defined by genetic studies (*13, 14*). On this basis, a simple model can be conceived in which coronin-1 reduces the efficiency with which proapoptotic complexes are delivered to the mitochondrial outer membrane (see the figure).

A surprise in this study is that coronin-1 has no role in forming the immunological synapse. Knowledge in this area is exploding, with recent demonstrations that the WAVE2 complex and HS-1 protein are essential activators of Arp2/3 for immunological synapse formation (*15*, *16*). The physiological role of coronin-1 appears to end when the T cell receptor is engaged with antigen, although coronin-1 accumulates in actin-rich projections in the periphery of the immunological synapse (*6*). It is possible that the role of coronin-1 is redundant with that of other factors that are recruited to the immunological synapse. These studies predict that distinct negative regulators of Arp2/3 will likely play an important role in T cell homeostasis after engagement of the T cell receptor. Negative regulators of Arp2/3 that control F-actin accumulation in the immunological synapse will likely play an important role in postactivation migration, energetics, and survival. The control of survival after activation is fundamental to immunological tolerance for prevention of autoimmunity and the formation of immunological memory—areas in which F-actin is likely to have a new role.

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GEOPHYSICS

Toward "Supervolcano" Technology

Time-lapse seismic tomography can provide detailed insights into magma movements in an active volcano and may help to predict volcanic hazards in the future.

Gillian R. Foulger

In addition to depicting the ultimate volcano-eruption horror story, the recent Discovery Channel/BBC coproduction "Supervolcano" speculates about what technology will be available to the geophysicist in 2025 to monitor active volcanoes. The result is a fictional Virtual Geophysical Laboratory that, when fed the right data, predicts eruption scenarios, thereby providing information to help guide civil emergency–response decisions. On page 821 of this issue, Patanè *et al.* (*1*) report a key step toward realizing such an advanced volcano-monitoring technology.

The authors have used time-dependent seismic tomography to study Mount Etna during its pre-eruptive and eruptive phases between August 2001 and January 2003 (see the figure). This method is analogous to CAT (computerized axial tomography) scanning in medical technology, except that earthquakes are used as energy sources and that regions of Earth are the target. In the present case, the region of interest is Mount Etna, a basaltic volcano in Sicily that is ~30 km in diameter and rises to ~3000 m above sea level.

The greatest challenge in this type of work is to obtain a sufficiently good earthquake data set. Patanè *et al.* combine data from multiple seismic networks to overcome this difficulty. They observe major changes in the ratio of seismic compressional to shear-wave speed (V_P/V_S) during the buildup to an eruption and during the eruption itself; these changes correlate closely with observed magma movements (*2*). Most notably, the authors map regions where V_P/V_S decreases, and attribute this decrease to the influx of magma that is rich in volatiles (SO_2, CO_2, and water vapor).

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Toward predicting volcanic hazard. Mount Etna emits plumes of ash on 29 October 2002. Patané *et al.* have used time-dependent seismic tomography to gain detailed insights into magma movements within this volcano. Further development of this method should help to predict volcanic hazards at Mount Etna and elsewhere in the future.

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This is the first report of time-dependent seismic tomography applied to an erupting volcano. It builds on earlier work of the same kind done in geothermal areas in California and Iceland and the Long Valley Caldera, California. But the seminal example of major changes in V_P/V_S comes from The Geysers geothermal area in northern California.

During the 1980s and 1990s, some 13,600 tons of steam per hour were extracted from The Geysers to generate electricity. As a result of this overexploitation, the reservoir became progressively depleted as pore water was replaced by steam. Repeat seismic tomography showed the steady growth of a reservoir-wide negative V_P/V_S anomaly that coincided with the steam-production zone. This anomaly was caused by the combined effects of the replacement of pore liquid with steam, the resulting decrease in pressure, and the drying of clay minerals. A remarkable series of snapshots showed the relentless growth of a volume of heavy depletion (*3*, *4*). The work helped to increase awareness of the nonsustainability of such high rates of fluid withdrawal. Production at The Geysers has now been reduced to sustainable levels. Time-dependent tomography is currently used to monitor the Coso Geothermal Area, southern California (*5*).

Time-dependent seismic tomography was first applied to a volcano in a study of Mammoth Mountain, a volcano on the rim of Long Valley Caldera, California. In 1989, an intense swarm of hundreds of earthquakes accompanied an injection of new magma into the roots of this volcano, and triggered the outpouring of some 300 tons of CO_2 per day from the volcano's surface. Several broad swaths of trees died as a result of high levels of CO_2 in the soil, and the CO_2 also presented an asphyxiation hazard to humans. A comparison of V_P/V_S tomographic images calculated for 1989 and 1997 showed changes that correlated well with areas of tree death on the surface above, and were attributed to migration of CO_2 in the volcano (*6*).

By showing that time-dependent seismic tomography can be used to monitor structural changes directly associated with a volcanic eruption cycle, Patanè *et al.* take a critical step toward developing a useful volcano-hazard-reduction tool based on seismic tomography. As with all good experiments, however, it ushers in new challenges. V_P/V_S is affected by several factors, including pore fluid phase, pressure, mineralogy, and fracture density. However, determining how each of these has changed when changes in only two quantities (V_P and V_S) have been measured is not possible and requires the addition of other kinds of data. Both theoretical advances and more data from different volcanoes are needed before the potential of the method can be fully assessed.

At present, monitoring of active volcanoes still rests mostly on relatively unsophisticated seismic networks and the monitoring of simple parameters, such as the numbers of earthquakes and the amplitude of harmonic tremor. Patanè *et al.* show that much more sophisticated methods can now be used. Some of these methods only need to be automated—a critical factor if they are to be useful in situations where information is needed on an hourly basis. It is hoped that this automation work will be pushed forward rapidly in the near future, putting us on track to realizing technological capabilities resembling those of the fictional Virtual Geophysical Laboratory by 2025.

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COMPUTER SCIENCE

Creating a Science of the Web

Tim Berners-Lee, Wendy Hall, James Hendler, Nigel Shadbolt, Daniel J. Weitzner

Understanding and fostering the growth of the World Wide Web, both in engineering and societal terms, will require the development of a new interdisciplinary field.

Since its inception, the World Wide Web has changed the ways scientists communicate, collaborate, and educate. There is, however, a growing realization among many researchers that a clear research agenda aimed at understanding the current, evolving, and potential Web is needed. If we want to model the Web; if we want to understand the architectural principles that have provided for its growth; and if we want to be sure that it supports the basic social values of trustworthiness, privacy, and respect for social boundaries, then we must chart out a research agenda that targets the Web as a primary focus of attention.

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When we discuss an agenda for a science of the Web, we use the term "science" in two ways. Physical and biological science analyzes the natural world, and tries to find microscopic laws that, extrapolated to the macroscopic realm, would generate the behavior observed. Computer science, by contrast, though partly analytic, is principally synthetic: It is concerned with the construction of new languages and algorithms in order to produce novel desired computer behaviors. Web science is a combination of these two features. The Web is an engineered space created through formally specified languages and protocols. However, because humans are the creators of Web pages and links between them, their interactions form emergent patterns in the Web at a macroscopic scale. These human interactions are, in turn, governed by social conventions and laws. Web science, therefore, must be inherently interdisciplinary; its goal is to both understand the growth of the Web and to create approaches that allow new powerful and more beneficial patterns to occur.

Unfortunately, such a research area does not yet exist in a coherent form. Within computer science, Web-related research has largely focused on information-retrieval algorithms and on algorithms for the routing of information through the underlying Internet. Outside of computing, researchers grow

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ever more dependent on the Web; but they have no coherent agenda for exploring the emerging trends on the Web, nor are they fully engaged with the emerging Web research community to more specifically focus on providing for scientists' needs.

Leading Web researchers discussed the scientific and engineering problems that form the core of Web science at a workshop of the British Computer Society in London in September 2005 (*1*). The participants considered emerging trends on the Web and debated the specific types of research needed to exploit the opportunities as new media types, data sources, and knowledge bases become "Webized," as Web access becomes increasingly mobile and ubiquitous, and as the need increases for privacy guarantees and control of information on the Web.

The workshop covered a wide range of technical and legal topics. For example, there has been research done on the structure and topology of the Web (*2*, *3*) and the laws of connectivity and scaling to which it appears to conform (*4*–*6*). This work leads some to argue that the development of the Web has followed an evolutionary path, suggesting a view of the Web in ecological terms. These analyses also showed the Web to have scale-free and small-world networking structures, areas that have largely been studied by physicists and mathematicians using the tools of complex dynamical systems analysis.

The need for better mathematical modeling of the Web is clear. Take the simple problem of finding an authoritative page on a given topic. Conventional information-retrieval techniques are insufficient at the scale of the Web. However, it turns out that human topics of conversation on the Web can be analyzed by looking at a matrix of links (*7*, *8*). The mathematics of information retrieval and structure-based search will certainly continue to be a fertile area of research as the Web itself grows. However, approaches to developing a mathematical framework for modeling the Web vary widely, and any substantive impact will, again, require a new approach. The process-oriented methodologies of the formal systems community, the symbolic modeling methodologies of the artificial intelligence and semantics researchers, and the mathematical methods used in network analyses are all relevant, but no current mathematical model can unify all of these.

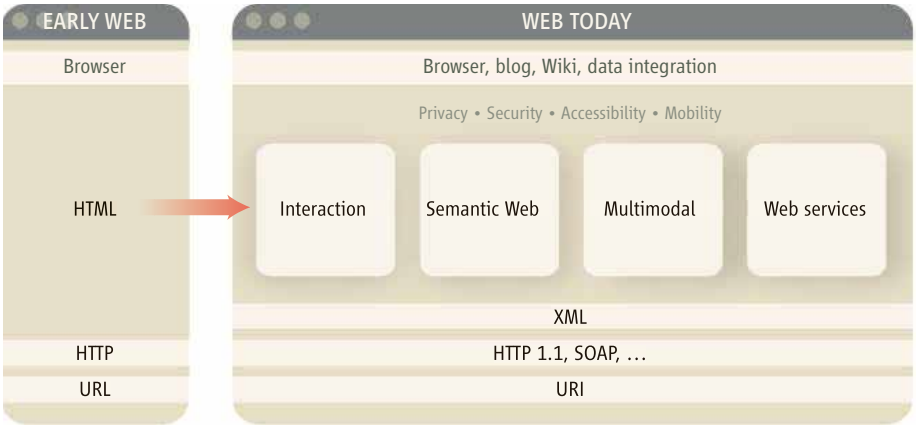
One particular ongoing extension of the Web is in the direction of moving from text documents to data resources (see the figure). In the Web of human-readable documents, natural-language processing techniques can extract some meaning from the human-readable text of the pages. These approaches are based on "latent" semantics, that is, on the computer using heuristic techniques to recapitulate the intended meanings used in human communication. By contrast, in the "Semantic Web" of relational data and logical assertions, computer logic is in its element, and can do much more.

Researchers are exploring the use of new, logically based languages for question answering, hypothesis checking, and data modeling. Imagine being able to query the Web for a chemical in a specific cell biology pathway that has a certain regulatory status as a drug and is available at a certain price. The engineering challenge is to allow independently developed data systems to be connected together without requiring global agreement as to terms and concepts. The statistical methods that serve for the scaling of language resources in search tasks and the data calculi that are used in scaling database queries are largely based on incompatible assumptions, and unifying these will be a major challenge.

Despite excitement about the Semantic Web, most of the world's data are locked in large data stores and are not published as an open Web of inter-referring resources. As a result, the reuse of information has been limited. Substantial research challenges arise in changing this situation: how to effectively query an unbounded Web of linked information repositories, how to align and map between different data models, and how to visualize and navigate the huge connected graph of information that results. In addition, a policy question arises as to how to control the access to data resources being shared on the Web. This latter question has implications both with respect to underlying technologies that could provide greater protections, and to the issues of ownership in, for example, scientific data-sharing and grid computing.

The scale, topology, and power of decentralized information systems such as the Web also pose a unique set of social and public-policy challenges. Although computer and information science have generally concentrated on the representation and analysis of information, attention also needs to be given to the social and legal relationships behind this information (*9*). Transparency and control over these complex social and legal relationships are vital, but require a much better-developed set of models and tools that can represent these relationships. Early efforts at modeling in the area of privacy and intellectual property have begun to establish the scientific and legal challenges associated with representing and providing users with control over their own information. Our aim is to be able to design "policy aware" systems that provide reasoning over these policies, enable agents to act on a user's behalf, make compliance easier, and provide accountability where rules are broken.

Web science is about more than modeling the current Web. It is about engineering new infrastructure protocols and understanding the society that uses them, and it is about the



The Web yesterday and today. (**Left**) The World Wide Web circa 1990 consisted primarily of text content expressed in the Hypertext Markup Language (HTML), exchanged via the hypertext transfer protocol (HTTP), and viewed with a simple browser pointing to a Universal Resource Locator (URL). (**Right**) Users of the Web now have a variety of top-level tools to access richer content including scalable vector graphics, the Semantic Web, multimodal devices (e.g., voice browsers), and service descriptions. These are expressed in extended markup language (XML), exchanged by newer protocols [e.g., HTTP 1.1 and SOAP (simple object access protocol)] and are addressed by uniform resource identifier (URI) schemes.

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creation of beneficial new systems. It has its own ethos: decentralization to avoid social and technical bottlenecks, openness to the reuse of information in unexpected ways, and fairness. It uses powerful scientific and mathematical techniques from many disciplines to consider at once microscopic Web properties, macroscopic Web phenomena, and the relationships between them. Web science is about making powerful new tools for humanity, and doing it with our eyes open.

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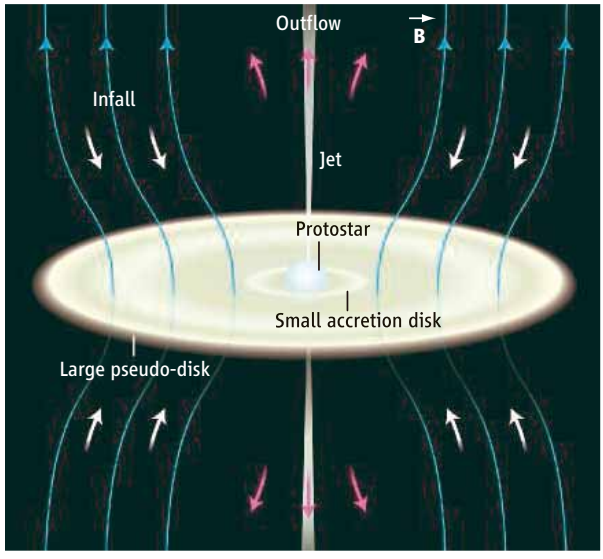
ASTRONOMY

Testing Star Formation Theory

Richard M. Crutcher

The importance of magnetic fields for the formation of stars, such as the Sun, is supported by measurements of polarized radio waves from dust particles near a newly forming star.

Understanding how stars form is one of the outstanding challenges of modern astrophysics. It has become clear that stars form from dense interstellar clouds of gas and dust, called molecular clouds because gas in such clouds is predominantly in molecular rather than atomic form. However, despite substantial progress in recent years, there remain fundamental unanswered questions about the basic physics of star formation. In particular, it remains unclear whether molecular clouds undergo rapid gravitational collapse as soon as sufficient matter accumulates to make the clouds gravitationally bound, or whether there is some mechanism resisting collapse that delays the process and introduces new star formation scenarios. The observational result reported on page 812 of this issue by Girart *et al*. (*1*) provides new data regarding this important scientific question.



Shaped by magnetism. Schematic diagram of a collapsing molecular cloud core with a strong magnetic field (**B**) showing the characteristic hourglass shape. [Adapted from (*4*)]

The "standard" model for the formation of low-mass stars such as our Sun has been that interstellar magnetic fields provide support against gravity in dense molecular clouds (*2*). In this picture, interstellar magnetic fields are "frozen" into interstellar matter by the small fraction of the gas and dust that is ionized. As material accumulates (due to the driving of flows by galactic spiral-arm shocks, supernovae explosions, the gravity of a galaxy, etc.), the magnetic field increases in strength as the gas density increases. After a molecular cloud accumulates sufficient mass to become self-gravitating, it will still not collapse and form stars because gravity is balanced by magnetic pressure.

If there were no other forces operating, molecular clouds would persist indefinitely and star formation would not occur. However, magnetic fields are frozen only into the ions of molecular clouds, not into the neutral gas and dust. The neutrals are therefore free to respond to gravity and collapse to form a much denser, gravitationally unstable core to the molecular cloud and eventually to form stars. However, as neutrals collapse through the ionized gas and dust, collisions with ions will occur. These collisions will greatly slow down the collapse rate, leading to molecular cloud lifetimes typically several orders of magnitude longer than the gravitational free-fall lifetime of a cloud.

In contrast to magnetically dominated star formation, the other extreme point of view is that magnetic fields are too weak to provide support against gravity. In this model, molecular clouds are intermittent phenomena, and the problem of cloud support for long time periods is irrelevant (*3*). Supersonic flows in the low-density turbulent interstellar medium produce regions of enhanced density. Star formation does not occur in every location where the gas is dense, but only in small volumes within clouds where sufficient mass accumulates to become self-gravitating. Collapse and star formation then proceed in that small fraction of the total cloud mass at a very rapid, free-fall rate.

In both models, the rate at which low-density interstellar gas is turned into stars is consistent with the observed star formation rate in the Milky Way Galaxy, about one solar mass per year. The strong magnetic field model achieves this result by setting the time scale for collapse of a dense molecular cloud much longer than the gravitational free-fall time. In the turbulent, intermittent model, only a small fraction of each molecular cloud actually becomes self-gravitating and forms stars. But the physical principles behind the two models are fundamentally different.

As a result, the two models make very different predictions that can be tested observationally. Simulations of molecular cloud formation and evolution carried out with weak magnetic fields show that the fields have a chaotic morphology, because the field lines are twisted by turbulence in the clouds. On the other hand, turbulence cannot twist field lines very much if the field strength is sufficiently strong. Magnetic field lines in dense, strongly magnetized clouds would then be roughly parallel. Collapse along the magnetic field is not impeded by the field, so cores are predicted to have a disk morphology. However, perpendi-

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cular to the magnetic field, the field impedes the collapse. As a dense cloud gradually collapses perpendicular to the field because of neutrals being driven toward the core by gravity, they will drag along the ions and the magnetic field. This will result in an hourglass structure to the magnetic field in a dense core (see the figure).

Interstellar magnetic fields can be observed because they produce polarization of the electromagnetic radiation emitted in or passing through the magnetized region. Irregular, spinning, paramagnetic interstellar dust particles will end up spinning about their short axis, and that short axis will be aligned with the magnetic field. An interstellar dust particle will therefore have a larger projected dimension perpendicular to the magnetic field than parallel to it. The dust will therefore radiate more strongly perpendicular to the magnetic field than parallel to it. By mapping the linear polarization of emission from dust particles in molecular clouds, it is possible to map the morphology of magnetic fields (projected onto the sky).

Such a map is what Girart *et al*. have produced for the low-mass star formation region NGC 1333 IRAS 4A. It is evident from the data that the magnetic field morphology is not chaotic, and it has the predicted hourglass shape. Their map shows gravitational collapse and future star formation caught in the act. The bent magnetic field lines have been drawn inward by the central gravity of the slowly collapsing molecular cloud. At the same time, the tension of the bent magnetic field lines resists gravity, slowing the collapse from the much faster free-fall rate. Moreover, they estimated the strength of the magnetic field and found that it was quite strong but just insufficient to prevent gravitational collapse, consistent with magnetically regulated star formation. This result strongly supports the strong magnetic field model of star formation, at least in this region, and provides important new data to astrophysicists working to understand how our Sun and the other stars form.

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MICROBIOLOGY

Breaking the Barrier Between Commensalism and Pathogenicity

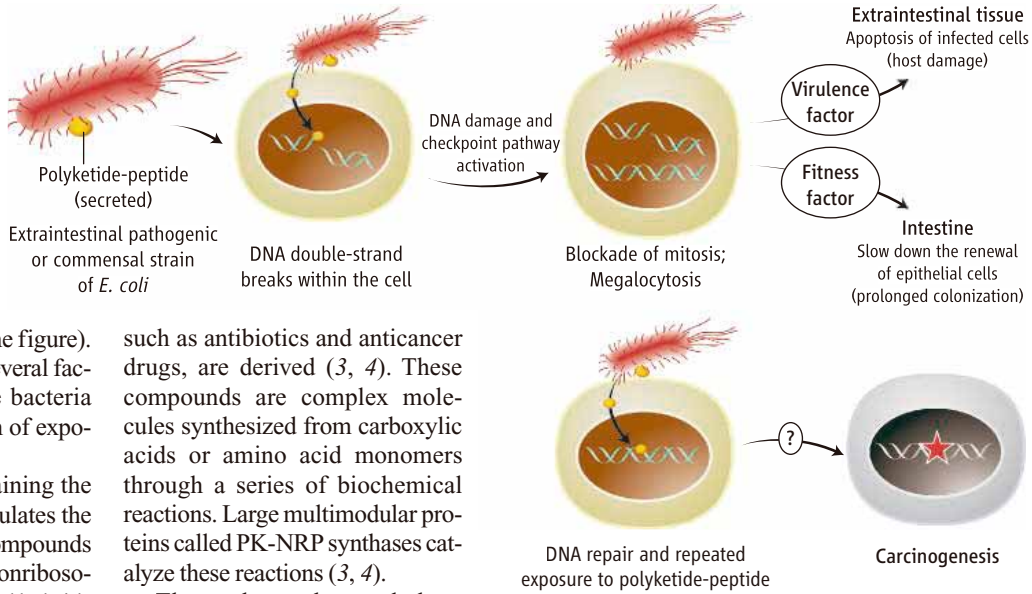
Harmless bacteria in the intestinal tract secrete a peptide that may enhance their survival. The same peptide causes disease when secreted by pathogenic bacteria outside the intestine.

Tetsuya Hayashi

Escherichia coli is a commensal bacterial inhabitant of the large intestine of humans and animals, but it also turns out to cause a wide range of diseases (*1*). On page 848 of this issue, Nougayrède *et al*. (*2*) report that certain *E. coli* strains harbor a set of genes that specify the biosynthesis of compounds that are either toxic to a host organism or can prolong bacterial survival and thus act as a fitness factor (see the figure). These opposing effects depend on several factors, including the location of the bacteria within a host organism and duration of exposure to the compound.

The large genomic region containing the gene set, called the "*pks* island," regulates the production of one or more hybrid compounds containing polyketides (PKs) and nonribosomal peptides (NRPs). PKs, NRPs, and hybrids of both are bioactive natural products that are widely produced in bacteria and fungi, from which many important therapeutic agents, such as antibiotics and anticancer drugs, are derived (*3*, *4*). These compounds are complex molecules synthesized from carboxylic acids or amino acid monomers through a series of biochemical reactions. Large multimodular proteins called PK-NRP synthases catalyze these reactions (*3*, *4*).

The authors observed that upon contact with cultured mammalian cells, certain *E. coli* strains induced megalocytosis, or gradual enlargement. They identified the *pks* genomic island as the underlying cause of the phenotype; inactivation of genes on the *pks* island prevented megalocyto-



Dual aspects of a bacterial toxin. A subset of *E. coli* strains, including both extraintestinal pathogenic and commensal strains, produce one or more polyketide–nonribosomal peptide hybrid compounds upon contact with a host eukaryotic cell. The compound damages DNA, which activates a pathway that leads to cell cycle arrest. Outside the intestine, the compound acts as a genotoxin; in the intestine, it functions as a fitness factor that enhances colonization. Long-term persistence of such strains in the colon may be involved in colorectal cancer development.

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sis, whereas introduction of the *pks* island conferred a laboratory *E. coli* strain an ability to induce megalocytosis. Although the authors did not succeed in isolating any of the compounds, the data indicate that such compounds induce DNA double-strand breaks in eukaryotic cells. Such DNA damage activates a cellular signaling pathway that leads to cell cycle arrest, the progressive enlargement of cell bodies, and eventually cell death (*2*). The PK-NRP hybrid compound thus acts as a genotoxin that induces genomic instability. As the genetic tool box has been poorly developed in most natural producers of PK-NRP compounds, the discovery of Nougayrède *et al*. will open a way to develop *E. coli*–based strategies to produce such hybrid compounds with improved or novel pharmacological properties.

Nougayrède *et al*. further noticed an interesting distribution pattern of the *pks* island in the *E. coli* species. *E. coli* consists of four major phylogenetic groups designated as A, B1, B2, and D. Among these, the *pks* island was found exclusively in the B2 group. Many strains belonging to this phylogenetic group cause extraintestinal infections such as urinary tract infections and septicemia. The PK-NRP hybrid compound can thus be regarded as a virulence factor of extraintestinal pathogenic *E. coli*. However, commensal strains from healthy people also possess the *pks* island. Nougayrède *et al*. show that one such commensal strain (Nissle 1917), which is nonpathogenic and widely used as a probiotic treatment for inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, produces functional genotoxin.

What would be the biological function of the genotoxin in commensal strains? The toxin may turn out to be either a fitness factor or a virulence factor, depending on the amount produced, the location or cell type of targets, and the duration of toxin exposure. In this regard, the hypothesis proposed by Nougayrède *et al*. is very attractive: Slowing the renewal of the intestinal epithelium by blocking the cell cycle may be a bacterial strategy to prolong colonization on the epithelium. Intestinal epithelial cells arise from stem cells that are located at the bottom of the crypts in the colon. These cells gradually differentiate during the upward migration, and terminally differentiated cells are shed into the intestinal lumen (*5*). Once a bacterium acquires an ability to control the turnover rate of epithelial cells to which it attaches, it could stay longer on the epithelium. The *E. coli* genotoxin can thus be regarded as a novel type of fitness factor that allows the bacterium to stably colonize in the colon. In agreement with this, the probiotic strain Nissle 1917 is an excellent colonizer, and *E. coli* strains belonging to the B2 group can persist longer in the colon than other groups (*6*).

Production of PK-NRP hybrid compounds by both normal inhabitants of intestinal flora and virulent strains of *E. coli* will change our current concept of pathogenicity and commensalism. By further investigating the biological role of these highly bioactive compounds, we will gain a more elaborated view of the molecular dialogue between bacteria and hosts. In addition, the genotoxic nature of the PK-NRP compound, which can act as a potential carcinogen, implies that chronic infection or long-term colonization with such genotoxin-producing *E. coli* strains may have an impact on tumor development. Colorectal cancer is principally a disease of industrialized countries, and strains belonging to the B2 group are predominantly isolated as commensal *E. coli* strains in these countries (*7*). The findings of Nougayrede *et al*. may also help to unveil the role of intestinal microbiota in the development of intestinal cancer, and more generally, to address the potential link between certain cancers and the production of bacterial toxins (*8*, *9*).

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BOTANY

Plant Cells CLEave Their Way to Differentiation

Rüdiger Simon and Yvonne Stahl

Two peptides with very similar amino acid sequences mediate short-range cell-cell signaling in plants. One suppresses and the other promotes the differentiation of stem cells.

Most plant cells are immobile and thus have to inform each other about their relative position through the exchange of chemical signals. Such cell-cell communication is particularly important during the development (differentiation) of stem cells to form specialized tissue cells. Two reports in this issue describe the isolation of small peptides that fulfill such short-range signaling functions (*1*, *2*). Both peptides are derived from precursor proteins belonging to the (almost) plant-exclusive CLE family and are very similar in amino acid sequence, but one promotes and the other suppresses stem cell differentiation during shoot and vascular development.

Plant stem cells are found in tissues called meristems, which are found at the tips of both the shoots and the roots (see the figure). Here, new cells are generated that enter differentiation paths to form specialized tissue cells. Stem cell identity in the shoot meristem is controlled by signaling to neighboring cells via the CLAVATA (CLV) pathway (see the figure, left). The known components of this pathway are CLV1 (a leucine-rich repeat receptor-like kinase), CLV2 (a leucine-rich repeat receptor-like protein), and the predicted ligand CLV3 (*3*–*5*). Stem cells secrete CLV3 to activate the CLV1/CLV2 complex in adjacent cells. This complex then represses the expression of the transcription factor WUSCHEL (*3*, *6*). Because this transcription factor is required for stem cell maintenance, increased CLV3 signaling induces stem cell differentiation.

The CLV3 protein is one of 31 proteins in the flowering plant *Arabidopsis* (a model organism for plant research) that contain the 14–amino acid CLE motif near their carboxyl terminus (*7*, *8*). Several other members of the CLE family can activate CLV signaling if they are expressed at sufficient levels in the meristem (*9*). Even short peptides con-

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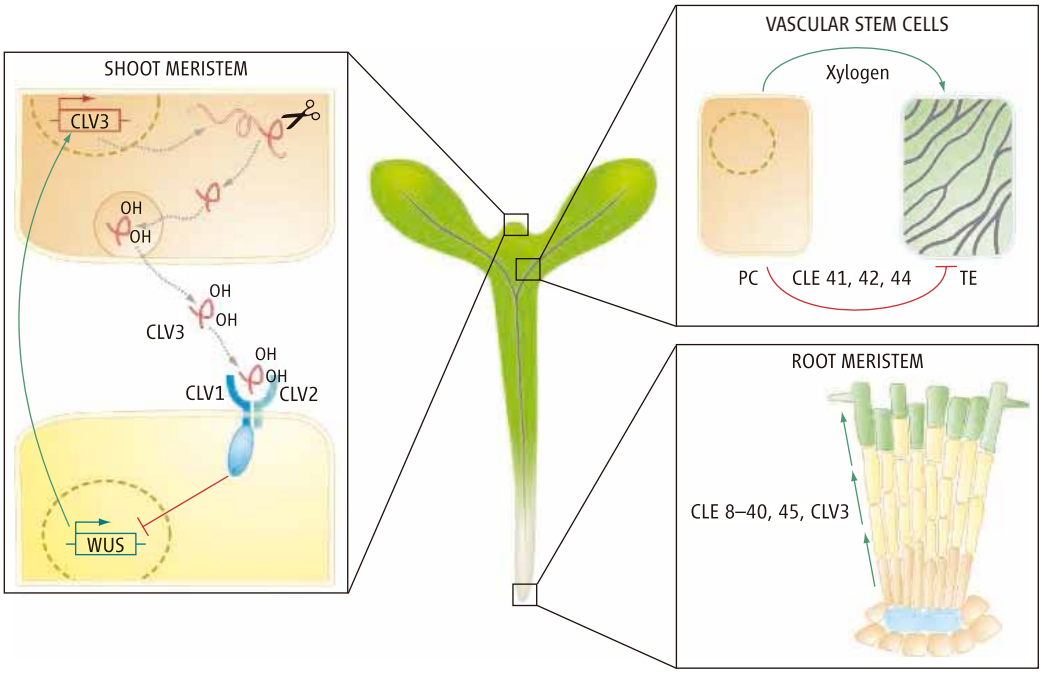

What it takes to be different. Effects of CLE peptide signaling on shoot and root meristems and on vascular stem cells (procambial cells). Stem cells are shown in orange, differentiated cells in green. (**Left**) Balancing stem cell numbers in the shoot. CLV3 is expressed in stem cells at the tip of the shoot meristem. The CLV3 precursor is processed into its active form, which contains only 12 amino acids of the CLE motif, as reported by Kondo *et al*. Extracellular CLV3 peptide is expected to bind to the CLV1/CLV2 receptor complex, leading to down-regulation of the transcription factor WUSCHEL, which in turn activates CLV3 expression and promotes stem cell fate. (**Top right**) Inhibition of xylem cell formation. Isolated mesophyll cells of *Zinnia elegans* first dedifferentiate, turn into procambial cells (PC) and then xylem precursor cells, and finally differentiate again into tracheary elements (TE). This differentiation process, mediated by the secreted factor xylogen, results in secondary cell-wall thickening and programmed cell death. Ito *et al.* isolated a small peptide homologous to CLE 41, 42, and 44 of *Arabidopsis* that inhibits this stage of differentiation in vitro, counteracting the xylogen differentiation signal. (**Bottom right**) Most other CLE peptides, including CLV3, can promote differentiation of *Arabidopsis* root meristem cells.

sisting only of the CLE motif retain this activity (*10*, *11*). But it has been difficult to determine the exact nature of the CLV3 peptide, because this peptide is expressed in only a few cells (the stem cells) at the meristem tip. Overexpression of CLV3 causes rapid stem cell loss and developmental arrest.

Kondo *et al.* have now acquired sufficient plant material for isolating the active CLV3 peptide. They did so by inducing leaf tissue of CLV3-overexpressing *Arabidopsis* plants to become callus tissue (which is not differentiated and can grow without restriction). They then applied matrix-assisted laser desorption/ionization time-of-flight mass spectrometry directly to this callus tissue. On page 845 (*1*), they report that the active CLV3 peptide consists of only 12 amino acids within the CLE motif and that it carries hydroxyl groups at two of the three proline residues. However, this hydroxylation is not required for signaling; its role may lie in controlling peptide stability.

To date, CLE peptides have been shown to promote the shift from the stem cell state to cellular differentiation in shoot and root meristems (see the figure, bottom right). On page 842, Ito *et al.* (*2*) show that CLE peptides can also inhibit differentiation.

The authors used plant vascular development as a model system to study the steps that control differentiation. The stem cells of the vascular system—the procambial cells—give rise to daughter cells (called xylem and phloem precursor cells) that subsequently differentiate into tracheary elements (see the figure, top right). This differentiation involves cell-wall thickening, loss of the nucleus, and ultimately cell death. To form a continuous tube system for liquid transport, the tracheary elements have to interconnect with each other, suggesting that their differentiation could be coordinated by short-range signaling.

This process can be studied in vitro using cultured leaf cells from *Zinnia elegans* plants. These cells differentiate into tracheary elements in the presence of the appropriate plant hormones. The resulting cells secrete factors into the medium that promote differentiation. One such secreted factor, xylogen, has previously been isolated from the cell walls of differentiating tracheary elements (*12*). Directed secretion of xylogen from one cell might coerce its next neighbor into vascular differentiation.

During the isolation of xylogen, Ito *et al.* also detected an inhibitory peptide, termed TDIF (tracheary element differentiation inhibitory factor), that suppressed the transition of procambial cells to tracheary elements and instead promoted cell division (*2*). The authors purified TDIF from conditioned medium and show that it is yet another hydroxyproline-carrying CLE peptide. Homologous CLE peptides from *Arabidopsis* have TDIF activity, but do not arrest root meristem development.

The identification of the active CLV3 peptide and of TDIF raises important questions: Where does processing of CLE peptides take place, which enzymes are involved, and how is it regulated? And how do processing and post-translational modifications contribute to determine the selectivity of CLE peptides for a specific signaling pathway? So far, CLE peptides have been shown to control the fates of shoot, root, and vascular stem cells. With 31 CLE peptides and more than 200 leucine-rich repeat receptor-like kinases as potential receptors in *Arabidopsis* alone, we have only seen the clef that starts the tune of differentiation.

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INTRODUCTION

When to Go, Where to Stop

THE ABILITY TO MOVE, AT SOME STAGE IN THE LIFE CYCLE, IS FUNDAMENTAL TO SUCCESS in life. Passive drift in water columns conferred a selective advantage for early life, offering an escape from starvation and genetic uniformity. Since then, organisms have evolved many ways to disperse and migrate in response to the pressures of finding resources, escaping predators, seeking out mates and suitable breeding grounds, and distancing themselves from family. Dispersal in its broadest sense means movement away from the birthplace. Strictly speaking, migration involves travel in a periodically and geographically predictable way, whether it occurs just once or many times. In this issue, *Science* deals with what we know, what we need to know, and how we are going to find out more about both of these movement types.

In plants, the spore, seed, or fruit is typically the unit of dispersal. Although the many morphological adaptations for their dispersal are known, until now, researchers have been unable to determine the distances traveled or the proportion of dispersal events that lead to seedlings. In one Perspective (p. 786), Nathan describes recent developments in the modeling and measurement of the long-distance dispersal of plants. A News story by Holden (p. 779) discusses the push to come up with a theoretical framework, not just for plants, but for all moving organisms. Organisms also disperse in reaction to changing habitats and climate. The Perspective by Kokko and López-Sepulcre (p. 789) discusses the selective forces affecting this ability in animals and how dispersal translates into range expansions and contractions. Kintisch (p. 776) describes the challenges for marine scientists assessing how climate change may affect oceangoing species.

Humans have been great dispersers. Colonizing new habitat has been a hallmark of human ecology over the past million years or so. In a Review (p. 796), Mellars considers recent advances in archaeology and genetics that are illuminating the controversies over the routes taken by ancient peoples in the colonization of Asia 40,000 to 60,000 years ago. Two Perspectives consider migration: Holland *et al.* (p. 794) focus on migrating insects, which tend to travel in established geographical patterns across several generations rather than returning to their birthplace, and Alerstam (p. 791) discusses the accumulating and sometimes conflicting evidence about the navigational mechanisms used by animals (particularly birds) in long-distance annual migrations. In a related Report (p. 837), Muheim *et al.* describe the role of polarized light at dawn and sunset in calibrating the magnetic compasses of migrating birds. A News story by Morell (p. 783) describes a new model that will clarify the mix of genes and environmental responses underlying successful bird migration.

As News stories by Blackburn and Holden (p. 780) and Unger (p. 784) point out, ingenuity and persistence are beginning to pay off in new techniques for following organisms, be they fish, crabs, jellyfish, rhinos, or polar bears. Thanks to these advances, the study of the ecology and evolution of movement is charging ahead and unearthing the challenges faced by organisms in dispersing and migrating in a world undergoing anthropogenic change.

– ANDREW SUGDEN AND ELIZABETH PENNISI

Migration and Dispersal

CONTENTS

See also related Report on page 837.

Science

CREDIT: CHRISTMAS ISLAND NATIONAL PARK

Giant migrant. In the Atlantic Ocean, leatherback turtles can travel 10,000 kilometers, taking routes that might be affected by a shifting climate.

CREDIT: GRAEME HAYS/INSTITUTE OF ENVIRONMENTAL SUSTAINABILITY

NEWS

As the Seas Warm

Researchers have a long way to go before they can pinpoint climate-change effects on oceangoing species

At 400 kilograms, the leatherback turtle might seem tough enough to withstand the vagaries of the ocean. This endangered seafarer voyages annually from the Caribbean to the North Atlantic and back in search of food. Yet it takes just a few degrees' change in the ocean's temperature for it to turn off course, says Graeme Hays, a marine biologist at the University of Wales Swansea in the U.K., who has tracked this species up and down the Atlantic for 4 years.

In finding that the leatherback is sensitive to water temperature, Hays and his colleagues have taken the first step toward assessing the potential impact of global climate change on this turtle. They join a growing number of marine scientists beginning to track the effects of a warming ocean on marine migrants, including commercially important species, such as cod and salmon.

Shifting migratory routes for endangered species such as the leatherback turtle could complicate what are already tricky conservation challenges for wandering sea creatures, says Lee Hannah of Conservation International in Washington, D.C.: "They're going to start turning up in places where there may not be conservation measures in place." Only by knowing the new routes and destinations of these animals, he adds, will protection measures be possible.

Researchers already know that for some migrating terrestrial species, birds in particular, climate change is making its mark. Systematic analyses of long-term data document shifting migration routes and earlier departures and arrivals, as well as suggesting a loss of synchrony between the migrant and its food source. Such hard evidence, however, has been lacking for the marine world. But with migrating sea animals showing up farther north than usual, "people are starting to ask, 'What [will happen] if the environment changes?' " says marine ecologist Patrick Halpin of Duke University in Durham, North Carolina.

On land ... and sea?

The current challenge for marine scientists is to make sense of largely anecdotal evidence about the effects of climate change on marine species. For example, in 1984, the northern boundary of migrating schools of jumbo squid (a smaller cousin to the giant squid) in the eastern Pacific was Point Conception off California; last year, the voracious predators were caught by Alaskan fishers. Similarly, marlins are appearing off the coast of Washington state, hundreds of kilometers north of their usual haunts. And sperm whales are frequenting the North Sea more than before. But are warmer global temperatures to blame?

For a growing number of species, mostly birds, the answer is yes. A 2003 meta-analysis of 1700 species—including some migratory species and a few marine animals—showed that warming temperatures have in recent decades moved species' range boundaries an average of 600 meters north per year. Behaviors are changing too. Warblers called blackcaps, for example, are wintering in the United Kingdom instead of Spain (*Science*, 21 October 2005, p. 419). Migrations are also happening sooner, with some European birds arriving 2 to 3 weeks earlier than 30 years before, and migratory bats are waking up from hibernation before their time. "For a while, it was a big deal" to have a documented impact of warming on a species, says ecologist Christopher Field of the Carnegie Institution of Washington, D.C. "Now there are examples all over."

Other studies have uncovered potentially serious consequences of such changes. In the 4 May issue of *Nature*, evolutionary ecologist Christiaan Both of the University of Groningen in the Netherlands showed that some populations of pied flycatchers flying from African wintering grounds to the Netherlands now arrive too late to catch their favorite local caterpillars. Warmer temperatures pushed their prey's peak emergence date up by 16 days, but the birds arrive only 1 week early, unaware that much of the food their young depend on will be gone.

Marine scientists have yet to find many similar examples of warming-induced changes, in part because they remain woefully ignorant about the distribution and movements of most sea creatures. "We don't know where these species are and what affects their migration, [and so we] don't know what impacts changes in temperature are going to

Sound Sightings

Scientists know much less about the lifestyles and travel habits of ocean dwellers than they do about most land animals. But newly developed systems based on acoustic sensing—one to track tagged fish and another to locate fish populations—promise to lay bare many secrets of the deep.

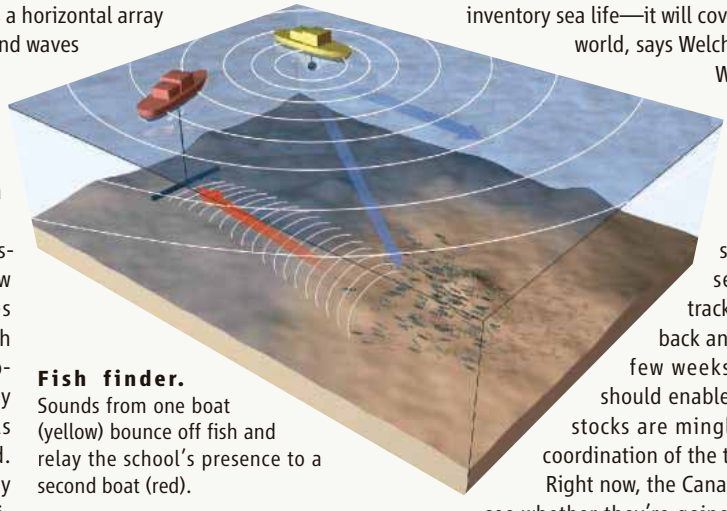
In late June, ocean scientists from about a dozen countries announced plans to set up a worldwide network of seafloor acoustic sensors, laid on continental shelves, to follow thousands of tagged fish. Another technology, soon to be tried out in the Gulf of Maine, relies on the acoustic properties of the relatively shallow coastal ocean to observe shoals of fish in real time.

In addition to providing insights into fish movement patterns, large and small, both approaches promise to improve fisheries management. "For fish that do not come to the surface, [it's] the only way to get precise locations and habitat use data," says Kim Holland, a marine biologist at the Hawaii Institute of Marine Biology, who tracks tiger sharks.

Ready to transmit. A biologist implants an acoustic tag into an anesthetized salmon smolt.

Traditionally, researchers use sonar to locate fish. From a boat, they send high-frequency acoustic signals that bounce off a fish's air-filled swim bladder and reveal its location. But sonar tells little about fish distribution because it only detects those in a 10-meter-wide column of water. Nor does it yield much information about fish movements, says ocean engineer Nicholas Makris of the Massachusetts Institute of Technology in Cambridge.

As an alternative, Makris has developed Ocean Acoustic Waveguide Remote Sensing. The technique exploits the fact that the shallow coastal areas of the continental shelf, where the ocean averages less than 200 meters deep, act as a "waveguide," allowing sound to bounce relatively unattenuated between the water's surface and the sea floor. The sensing strategy requires two boats. Hanging off one, a vertical array of speakers emits low-frequency chirps that make the surrounding water vibrate "like a guitar string," says Makris. The other boat, several kilometers away, deploys a horizontal array of hydrophones that pick up sound waves deflected by the fish. The result is a constantly shifting two-dimensional image—like a weather radar image—of fish densities over a huge area (*Science*, 3 February, p. 660).



Fish finder. Sounds from one boat (yellow) bounce off fish and relay the school's presence to a second boat (red).

When Makris tested the system in 2003, off the coast of New Jersey, he and his colleagues were able to detect groups of fish over thousands of square kilometers. They watched minute by minute as schools and shoals formed, divided, and scattered. The technology is "extremely exciting, as it allows the identification of patterns of fish density over a very wide range of scales," says zoologist Iain Couzin of the University of Oxford, U.K. It could enhance data-gathering 1000-fold. This fall, Makris's group will head for the Gulf of Maine to help the National Marine Fisheries Service in its annual survey of North Atlantic herring.

The proposed Ocean Tracking Network would use a different technology, underwater acoustic receivers and small acoustic tags, to tail far more fish and a greater variety of them far more cheaply than current satellite or ship-based tracking programs allow, says coordinator Ronald O'Dor, a biologist at Dalhousie University in Halifax, Canada. Satellites, for example, can track radio signals only when an animal is at the surface, and the flashlight-sized transmitters can be attached only to large fish and marine animals.

In contrast, the new network could track submerged animals, big and small, and would not require ships or satellites to pick up the signals. The test bed of the proposed network has been an array of underwater acoustic receivers called the Pacific Ocean Shelf Tracking Project (POST). Two years ago, fish biologists captured 2000 juvenile wild salmon spawned in rivers in Canada and the United States, surgically implanted them with small acoustic transmitters, and let them loose to make their way to the Pacific. There, the fish were monitored by soda can–sized sensors deployed in six 20-kilometer-long "listening lines" perpendicular to a 1500-kilometer stretch of Canadian coastline. The fish tags—acoustic equivalents of supermarket bar codes—last from 6 months to a couple of years. Fingernail-sized ones transmit only an ID, but larger tags daily record location, temperature, and depth and can dump the information when they come near a receiver, says oceanographer David Welch, head of POST. With such data, it may be possible to find the cause of a decline in salmon that hatch in the Columbia River, notes Ben Zelinsky of the Bonneville Power Administration in Portland, Oregon.

The international network will ultimately require about $167 million to set up. Part of the Census of Marine Life—a global 10-year initiative to inventory sea life—it will cover 14 ocean regions throughout the world, says Welch.

Welch, who runs Kintama Research Corp. on Vancouver Island in British Columbia, Canada, says the network could clear up fisheries management questions. For example, "European" and "American" stocks of bluefin tuna are managed separately. Yet, he notes, satellite tracking has shown that fish can "move back and forth across the Atlantic in just a few weeks." The Ocean Tracking Network should enable scientists to determine how those stocks are mingling, which should lead to better coordination of the tuna fisheries, he says.

Right now, the Canadians are on tenterhooks waiting to see whether they're going to get the $32 million they have applied for from the Canadian government to get the project rolling; many other countries whose researchers are involved in the effort are also being asked to chip in. "One of the most difficult things," says Welch, is persuading people "just how desperately we need this information—because we've never had it." **–C.H.**

CREDITS (TOP TO BOTTOM): PACIFIC OCEAN SHELF TRACKING PROJECT; C. BICKEL/*SCIENCE*

have on them," says Duke University marine biologist Michael Coyne.

In work begun in 2002, Hays took a stab at answering this question by outfitting nine leatherbacks with radio recorders that transmit position, water temperature, and other data to satellites. He confirmed observational records that they are venturing higher into the North Atlantic compared to historical trends. Hays had presumed that the turtles migrated northward as far as it took to find rich reservoirs of jellyfish and other foods. But the work, which is reported in the July issue of *Global Change Biology*, shows that these lumbering beasts go north only until they hit 15°C water, regardless of where their prey are. He calls that "a total surprise." Leatherback migration limits, he says, "are driven by temperature, not food."

Critics point out that nine turtles are very few upon which to base a theory, and that the temperature-migration link says little about how climate change might ultimately affect the survival of the leatherback species. Still, the work "is certainly pointing in the right direction as to where the field has to go," says behavioral ecologist Julian Metcalfe of the British Centre for Environment, Fisheries, and Aquaculture Science in Lowestoft, U.K.

Heat is on. Field and lab studies disagree about the ability of cod to thrive in warming temperatures.

Adrift with data

Until recently, finding and following an oceangoing migrant was a big stumbling block for marine scientists. Today, better, cheaper technology is slowly allowing marine migration work, such as Hays's, to come into its own (see sidebar, p. 777). A satellite tag for a sea turtle still costs the same $5000 it did years ago but now lasts months longer, transmits several times a day instead of once every few days, and records data that include dive times and pressure, says ecologist Brendan Godley of the University of Exeter, U.K. Coin-sized data recorders log months of complex data for fish and are retrieved when fishers catch the animals, and new tags that provide coordinates from the Global Positioning Satellite network offer better spatial resolution and battery life.

However, the flood of data from these improved instruments is "raising more questions than answers," Godley says. He used satellite tags in 2004 to figure out where loggerhead turtles foraged after leaving their Cape Verde, Africa, home. Godley says he was amazed to learn that 7 of 10 tagged turtles stayed out at sea for the whole foraging season—a then-unknown behavior that may or may not change as the oceans warm.

Other tagging studies are showing that the actions and preferences of captured or lab-reared animals don't always reflect behavior in the wild. In the lab, for example, the ideal water temperature for Atlantic cod is between 13° and 15°C. But cod surgically implanted with data recorders and released back into the ocean have revealed that the fish actually live for months in shallow water warmer than 17°C, according to recent unpublished work by Metcalfe. Researchers might be underestimating certain species' environmental flexibility, another important consideration for determining whether climate change is harmful, he says.

Another significant challenge for researchers hoping to assess the impact of climate change on migrating marine animals is that historical records are sparse. There are storerooms full of fishers' catch records, but those "are only loosely related to real changes in population size," says population biologist Camille Parmesan of the University of Texas, Austin. Fishers tend to be inconsistent in how they calculate their catches and sometimes fail to record when and where certain fish were caught, she explains.

Where long-term data exist, climate change seems to be proving important. The Continuous Plankton Recorder survey, started in 1946, maintains population records on these key microorganisms at the bottom of the ocean's food chain. The plankton are collected in screens towed voluntarily by merchant ships in the North Atlantic and North Sea. A 2003 analysis of the 5 decades' worth of data, by researchers from the Sir Alister Hardy Foundation for Ocean Science in Plymouth, U.K., suggested that rising North Atlantic temperatures were linked to a change in plankton distribution, leading to what amounted to a northward shift in the southern boundary of salmon.

"Historic records are so valuable when you start thinking about [climate] change," Halpin says. "There are so many things like that we wish had been done."

As their analyses are getting more sophisticated, marine ecologists considering the impact of climate change are seeking more interdisciplinary approaches and combining different kinds of data more extensively. Godley has recruited a climate modeler to help him come up with realistic scenarios with respect to physical changes predicted for the marine environment. Duke University marine scientists are helping other researchers take a more comprehensive approach to migration studies by compiling marine mammal, seabird, and sea turtle data in a geographically organized database, complete with data on climate and ocean-floor topography. The database will be part of the new online Ocean Biogeographic Information System, which will be publicly accessible.

Such interdisciplinary approaches are paying off. By analyzing the locations of hake, a commercially harvested Pacific fish, with respect to a subsurface Pacific current called the poleward undercurrent, fisheries oceanographer Vera Agostini of the Pew Institute for Ocean Science at the University of Miami in Coral Gables, Florida, has found that schools of this species may vary their migration patterns depending on the strength of the current. During an El Niño year, when the northward current was robust, the hake were "getting on the highway," by concentrating in the strong flow's edge, says Agostini. But in 1995, a

non–El Niño year with a weaker current, the fish were found within the current less frequently, she will report in the *Canadian Journal of Fisheries and Aquatic Sciences*. Because climate change is expected to affect ocean flow and the frequency of El Niño, Agostini predicts it might in turn affect the hake's migration behavior.

There's a natural tendency to assume that climate change will have negative impacts on marine species, but some scientists caution that that might not be the case for every creature. If cod can thrive at higher-than-expected temperatures, for example, they may adjust just fine to a warming ocean, at least for a while. Turtles may have greater access to one of their favorite foods, adds Hays, noting that his 3-year aerial study of the Irish Sea found huge assemblages of jellyfish, ready for the picking by leatherbacks should that water get warm enough—above the proposed 15°C cutoff—for the turtles' liking.

Whether turtles, salmon, or other sea creatures will fare better or worse in a warming world is unanswerable at this point, admit marine scientists. But Halpin urges his colleagues to take on these questions. Determining how climate change affects marine migrants, he says, "is the next horizon."

–ELI KINTISCH

NEWS

Inching Toward Movement Ecology

With ever more data coming out on migration, dispersal, and other movements, a few researchers say it's time for some synthesis

For centuries, researchers have sought to understand when, why, and how various species crawl, swim, fly, float, or hoof it to new locales. That work has led to maps of migration routes and details about dispersals.

But few biologists have tried to fit these data into a big picture of movement in general, says Ran Nathan of Hebrew University in Jerusalem. Under the auspices of a new discipline called "movement ecology," he and others are beginning to derive testable hypotheses about the mobile behaviors of animals, microbes, and even the seeds of plants. Their goal is to join empirical work to theories and to build models that fill in gaps in our understanding of movement—be it over millimeters or continents or by groups or individuals—in the natural world.

Nathan and his students, for example, have been analyzing how birds fly and seeds disperse, looking for common ways that both plants and animals react to wind. Colleagues elsewhere are building computer models showing how very different species of animals, such as guppies and bees, may follow similar rules while on the go. These researchers are also looking at how laws of physics can help explain group behavior.

Movement ecologists contend that their work will have practical applications. Wayne Getz, an applied mathematician at the University of California, Berkeley, says new, more fine-grained methods for studying movement will help researchers understand the spread of bovine tuberculosis in moving buffalo herds in South Africa. Or conservation biologists may find that the proliferation of invasive species, be they viruses, weeds, or goats, is governed by some common rules that, once understood, could be used to quash an invasion. "This is an important emerging field," says Paul Barber, a marine biologist at Boston University.

At first glance, Nathan and colleagues seem to be simply applying a new label to what some researchers have been doing for years. Yet they may succeed in drawing attention to an underappreciated component of ecology, says Daniel Janzen, a tropical ecologist at the University of Pennsylvania. "Practically every field biologist I know deals with movement ecology all the time," he explains. "But an awful lot of biologists conveniently trim that out of their way of thinking to make their problems simpler." For instance, he says, when researchers can't find a particular butterfly where they expected, they tend to assume that the species is dormant or has declined and don't consider that its members may have simply moved on.

Nathan is hoping to create new sets of assumptions. With a grant from his university's Institute for Advanced Study, he has invited a group of scientists, with expertise in areas as diverse as zebra migrations and the mathematical and genetic analysis of pollen flow, to spend all or part of the next academic year in Jerusalem hammering out the new field.

Defining it is difficult at this point, says Marcel Holyoak, an environmental scientist at the University of California, Davis, and one of Nathan's recruits. "The core of movement ecology is seeking a unified theoretical framework for studying movement, and such a framework is not yet available." The thinkfest in Jerusalem is supposed to create that framework, then move on to develop data sets that integrate information collected on different species, at different scales, and on

Continued on page 782

CREDIT: JORGE RODRIGUES

Frequent flier. Even the versatile bee-eater is at the mercy of winds.

TAG TEAM

Animals are often leaving researchers in the dust. A bird flies off. A crab scrambles under a rock. A whale dives and swims away. Yet migrations, dispersals, and other types of movements are important components of an organism's life history and of an ecosystem's health. To keep an animal in their sights, so to speak, researchers increasingly rely on technological innovations as diverse as miniature radio transmitters and remotely guided submersibles. As the stories on these two pages show, the technologies are imperfect, but nonetheless, ecologists are finding their way toward a greater understanding of the mobile species they study.

CREDIT: CHRISTMAS ISLAND NATIONAL PARK, AUSTRALIA

Crab Walk

Every November, the slopes of Christmas Island, an Australian territory in the Indian Ocean, develop a deep red rash. With the start of the monsoon rains, 45 million adult red land crabs (*Gecarcoidea natalis*; above) seem to boil out of the ground for a 7- to 10-day, 8-kilometer trek to the coast. At the ocean, the crabs mate. Then the males embark on the return journey. The females follow once they have incubated and laid their eggs. The larvae are confined to the salt water for 3 weeks, after which they emerge as miniature adults that head into the hills.

Through sheer force of numbers, the migration is hard to miss. The challenge has been to pin down the crabs' exact migration routes. To track the crustaceans, physiologist Steve Morris of the University of Bristol, U.K., puts small radio transmitters on the crabs and follows behind with a hand-held receiver.

The job can be "a nightmare," Morris says, because of the island's topography and the crabs' habitat. "Christmas Island is shaped a bit like a tiered wedding cake," he says. "If a crab has moved over the top of one of the tiers, then we can't pick up its signal." The crabs' jungle environment also doesn't help. Signals are bounced around by dense, wet foliage, and pinnacles of rock often disturb signal transmission.

Nonetheless, through these and other tracking efforts, including using paint to color-code the shells of crabs from different locations, Morris has shown that the animals travel in a surprisingly straight line—often up and over bumps instead of around them. He has also discovered that the crabs head to a specific beach, then backtrack to their original jungle haunts. Using the radio transmitters, Morris is also following individual crabs during their daily routines once they are back in the jungle. In this way, he's learning how their bodies change as they shift from having to deal with the stresses of jungle life to the challenge of their annual journey.

–LAURA BLACKBURN

Eye in the Sky

Last fall, using a combination of eyelash adhesive and Krazy Glue, a team led by Martin Wikelski of Princeton University attached tiny radio transmitters to the abdomens of 14 migrating green darner dragonflies (*Anax junius*; lower right). Then, using receiver-equipped cars and small planes, the scientists tracked the insects through New Jersey as they made their way south. The 300-milligram transmitters worked for 12 days, sending information about the speed and timing of the bugs' travels as well as temperature and wind speed.

Now, Wikelski wants to use these "mini" tags to follow small flying animals around the world, via satellite. So far, satellite tracking has been used only on large animals, such as caribou or whales, wearing powerful transmitters with long-lived batteries. But miniaturization of the technology is now making it realistic to try to track some of the 6 billion songbirds—as well as countless bats and insects—that migrate between continents each year.

In a project he calls Icarus, Wikelski envisages placing a refrigerator-sized satellite in low Earth orbit. It would pick up signals from migrating birds, such as wood thrushes, warblers, and finches, outfitted with 2-gram sensors. It's also possible that Icarus's receivers could piggyback onto another low-orbit vehicle.

Either way, it's all very pie in the sky at the moment, says electrical engineer George Swenson of the University of Illinois, Urbana-Champaign, who has been advising the project. After all, Project Icarus lacks any funding. Among the possibilities is the United Nations, as Icarus has the potential to track pests such as desert locusts. Wikelski admits it may take a decade to get the idea off the ground. But Smithsonian Institution ornithologist Peter Marra says, "It would be tremendous" to be able to track migratory birds this way. **–C.H.**

CREDIT: MARTIN WIKELSKI/PRINCETON UNIVERSITY

CREDIT: DIANE COWAN

In the Deep Blue Ocean

Radio transmitters won't work on swimming animals that never surface, but sonar does the job just fine. In Maine, marine biologist Diane Cowan of The Lobster Conservancy in Friendship, along with volunteers and fishers, uses underwater hydrophones to pick up signals from sonar tags attached to lobsters (*Homarus americanus*; left). In June, Cowan reported that her team had tagged 191 lobsters and reestablished contact with 82% of them at least once over the following year.

In analyzing these new data, she's found that not all wintering female lobsters head to deeper, warmer water to incubate their eggs. "This was a surprise," says Cowan, as eggs need at least 3°C water to develop. As expected, large females did migrate out to sea, traveling an average of 58 kilometers. But small females stayed in shallow water, which sometimes dropped below freezing. These waters warm up sooner in the spring, which may compensate for the females' failure to move, she points out. **–L.B.**

Jellyfish on the Run

If you can't tag 'em, then chase 'em. That's Bruce Robison's new motto. A marine biologist at Monterey Bay Aquarium Research Institute in Moss Landing, California, he has tried to follow jellyfish by gluing, surgically implanting, or even feeding acoustic transmitters to the animals, without much luck. He has now turned to remotely operated vehicles (ROVs; right). Because ROV pilots have a hard time following the jellies for very long, Robison has recently teamed up with Stephen Rock's aerospace robotics lab at Stanford University in Palo Alto, California. At the lab, Jason Rife has come up with a software-driven camera system that can lock in on the quarry and maneuver the ROV without human intervention. The software keeps the ROV away from a jelly and adjusts the ROV's course as needed. To date, the group's record for keeping a jelly in sight is 89 minutes.

Stanford graduate student Aaron Plotnik is developing new software that will be able to track faster, smaller jellies, says Rock. Robison envisions autonomous robots that follow the jellies for weeks and record temperature and salinity. And then, he says, we can finally answer the simple question, "How deep do jellies go?" **–L.B.**

CREDIT: KIM FULTON-BENNETT/MBARI

CREDIT: NIGEL CATTLIN/ PHOTO RESEARCHERS INC

On the Radar Screen

A beetle's world often extends hundreds of meters into the air. When it and other insects travel in the upper air currents, they are well beyond the reach of nets, traps, and other traditional tools of entomologists. "We need to [reach] into the atmosphere; otherwise, we are missing out on an important part of their ecology," says Jason Chapman, an entomologist at Rothamsted Research in Hertfordshire, U.K. His solution: vertical radar (lower left).

Rothamsted Research's radar system, one of a handful of vertical radars currently operating around the world for scientists, has been sending a beam skyward 24/7, rain or shine, since 1999. The radar beam is cone-shaped, just a few centimeters wide when it leaves the transmitter but covering a swath a few meters in diameter at the team's recording limit, around 1200 meters high.

In the 1970s, physicist Glen Schaefer, then at Loughborough University in Leicester, U.K., pioneered the use of vertical radar for tracking insect pests, such as desert locusts. But there was no automated processing of the incoming signals, and instead, "people had to sit by the radar screens and take photos," Chapman says. "It was very time-consuming."

Now, however, computers do most of the work. The returning radar signals are continuously recorded and analyzed using specially designed software that determines the size, shape, flight speed, and direction of insects passing through the vertical radar beam. Chapman combines his radar analyses with long-term data on wind speed and direction to piece together an insect's migration path and predict where it came from.

He and his colleagues have discovered that some insects disperse much more widely than thought. Last year, they showed that a local carabid beetle (*Notiophilus biguttatus*; top left), which feasts on agricultural pest species, doesn't keep to a single field as was previously believed. Instead, the beetles showed up in aerial traps and could be detected up to 400 meters high in the radar beam, indicating that carabids were on the move. **–L.B.**

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Continued from page 779

different types of movements. Ecologist Peter Smouse of Rutgers University in New Brunswick, New Jersey, says scientists might glimpse a hitherto unseen "bigger picture" of, say, rare colonization events, if provided with a data set that brings together varied examples of this phenomenon, such as an exotic plant that had been transported by freighter across the ocean, or pollen from a genetically engineered plant that was blown to a new field. Nathan says scientists will then use such data sets to test new ideas about the role of physiology, evolution, behavior, and environmental forces in shaping when and how organisms move.

Modern technology is providing the flood of information necessary for the birth of movement ecology. Advances in the analysis of stable isotopes of common elements now allow researchers to tell where a bird has been since its last molt because the isotopes—variants with different atomic weights that are specific to particular latitudes—get into the food chain and eventually show up as chemical geographical signatures in feathers. Better genetics techniques are helping clarify the dispersal history of populations of many different species. Global positioning systems and the miniaturization of animal-tracking tags are making it possible to collect migration and dispersal data in unprecedented detail over long periods (see p. 780).

Stubborn. Milk thistle won't yield to just any puff.

And thanks to greater computing power and prowess, researchers can manipulate these data in new ways. As a result, scientists can "start to make what now seem to be impossible comparisons," says Nathan. Princeton University mathematician Simon Levin, who looks at the movements of a wide variety of organisms—from phytoplankton to locusts—and of the influenza A virus, says the approach will lead to rules that govern movement "across scales of space, time, and organizational complexity."

Levin and Iain Couzin of the University of Oxford, U.K., who is moving to Princeton University in the fall, have already used data on several species of fish and insects to build a generic model that describes group movements. The model shows that only a tiny number of "informed" individuals—that is, those familiar with a food source or migration path—are required to bring around the whole group, they reported in the 3 February 2005 issue of *Nature*. These few are somehow able to get "naïve" members to "reconcile the tendency to clump together" with the tendency to follow those in the know, says Couzin.

The researchers found that the larger the group, the smaller the proportion of leaders required. In the case of bees, this behavior likely evolved as a more efficient way to transfer information: Only a few individuals need to take the time to observe the waggle dance, and the rest just follow along. The duo also figured out rules by which a group reaches consensus when the informed individuals differ with one another on the course to take. They are now testing these ideas with data on other fish species as well as on human groups.

In some cases, the models employed by movement ecologists are coming from other disciplines. In one study, Couzin, with Jerome Buhl of the University of Sydney in Australia, turned to theoretical physics. They used a model that predicts the behavior of magnetic particles to forecast the behavior of marching locusts (*Science*, 2 June, p. 1402).

Couzin and Buhl filmed a band of locusts circling a dome in the lab, which gives the locusts the impression they are in an endless swarm. When the marching locusts reach a certain density, their movements change from a chaotic to a highly ordered state, and they suddenly align with the paths of their nearest neighbors, the researchers reported. At densities of 24.6 per square meter, the insects began to march together, behaving like magnetic particles, which also start to align at increased densities. But the group still occasionally made rapid, spontaneous changes in direction without losing group cohesion. By the time densities surpassed 73.8 per square meter, however, the locusts surged along as one, with no direction changes.

While some researchers push movement ecology's frontiers forward in the lab and at the computer, Nathan has his students out in the field. For example, graduate student Nir Sapir marked bee-eaters and tracked their spring migration along the Negev and the Arava Valley in southern Israel. He mounted tiny radio transmitters on birds' backs, followed them in a car, and trapped the birds along the way. In all, he and his team recorded the paths of 11 birds, showing that even for a bird of the bee-eater's nimble flying abilities, courses and distances depend strongly on wind conditions—reminiscent of windswept seeds. In both the seeds and the bee-eaters, there's a "relative lack of control of their movements and [a] clear dependence on external conditions," Nathan says.

In other work, graduate student Ana Trakhtenbrot and colleagues put milk thistles in a wind tunnel to see exactly what force it would take to dislodge the seeds. Unlike a dandelion, the milk thistle is not at the mercy of the lightest puff—it resists all but stiff winds. The results show that the thistles, like birds, have some, but not total, control over how the wind affects them. Cataloging similarities in the movements of plants and animals such as bee-eaters and thistles can open doors to new ways of looking at the natural world, says Nathan.

Couzin points out that once movement ecologists hammer out common principles among diverse types of motion, their efforts may prove relevant to other fields, even reaching into the social sciences. For example, the way many viruses spread—accelerating as the number of individuals infected increases—in some respects resembles the way information is disseminated. In fact, Couzin says, one of his colleagues tried putting short-range sensors on people at dinner parties to work out social networks and the potential spread of information.

But for biologists, the relevance of this approach is in how it will influence their thinking, says Janzen: Movement ecology "might make it more fashionable or legitimate to try to add the movement part [back] into biology." **–CONSTANCE HOLDEN**

CREDIT: RAN NATHAN

CREDIT: ROLF NAGEL

Cued to fly. A northern wheatear is genetically programmed to take a refueling stop on a long-haul migration but can adjust its stay as needed.

NEWS

Arduous Journeys

Researchers assess the balance between instinct and adaptability that makes long-distance migrations doable

It may be just a small songbird with gray feathers and an eye-catching white rump, but the northern wheatear (or less politely, northern white-arse) is the consummate transoceanic migrant. And thanks largely to the efforts of Franz Bairlein of the Institute of Avian Research in Wilhelmshaven, Germany, it's an up-and-coming star in migratory biology.

Northern wheatears (*Oenanthe oenanthe*) boast the largest breeding range—covering nearly half the planet—of any migrating songbird. In the spring, some subspecies nest in northern Africa. Others reproduce in the northern polar regions from Alaska to Siberia. Some populations head to Scandinavia, Greenland, Iceland, and northeastern Canada.

At the end of the summer, however, they all converge on the savannas south of the Sahara Desert—with each population flying considerably different routes. One Moroccan subspecies migrates a mere 300 kilometers to southern Morocco. At the other extreme, another subspecies travels from eastern Canada to Africa, crossing the open ocean and Greenland's ice fields before refueling and turning southwest in the United Kingdom.

By observing wheatears from different places, the researchers are finding much-needed answers to questions about how the birds respond to environmental cues—a factor that may be key to the species' survival as the global climate changes. "They're an excellent model bird for many [migration] questions," says Wolfgang Wiltschko, an ornithologist at the University of Frankfurt in Germany.

For decades, researchers depended on various species of short- and long-distance migratory European warblers for many migration studies. Over the years, ornithologists have demonstrated a strong genetic component to migration. In 1968, using leaf warblers (*Phylloscopus* sp.), Eberhard Gwinner of the Max Planck Institute for Ornithology in Andechs, Germany, showed that these nocturnal travelers had an innate drive to migrate—restlessly hopping through the night even in captivity—when it was time to move. Gwinner and others have since demonstrated that these warblers also have built-in compasses and navigation systems—they instinctively know where to go—as well as calorie counters that help them determine how much to stock up along the way.

Most of these findings came from studies of warblers in the lab. But when scientists attempted to do field experiments to find out how the birds adjust to change, they were thwarted by the warblers' preference for dense, shrubby habitats. Wheatears, in contrast, are open-country birds, eating and cavorting in plain view. In addition, they can be bred in the lab, says Bairlein.

An environmental physiologist, Bairlein started studying northern wheatears in 1998. As part of that work, he and his students have been using the wheatears to explore how much the birds stoke up on insects and berries for their flights, one of the many physiological and ecological challenges of their various journeys.

In 2001, Bairlein's students Volker Dierschke and Julia Delingat discovered a difference in how two subspecies spend their time refueling on the North Sea island of Helgoland during their spring migrations north. One, *O. o. oenanthe*, needs to fly only an additional 50 to 500 kilometers from the stopover island to its Scandinavian breeding grounds. In contrast, the natal homes of *O. o. leucorhoa* are in Greenland and Iceland, 2500 and 1000 kilometers away, respectively.

The team banded approximately 250 birds of each subspecies at Helgoland and tracked their feeding behaviors and departure times. The Scandinavian birds treated the island like an In-N-Out Burger stand, merely touching down for a quick snack. But those wheatears heading to Greenland and Iceland settled in for a good daylong feed.

Bairlein's team is complementing its field study with comparisons of these two subspecies in the lab. The researchers are finding further support for a genetic drive to bulk up in the birds with the farthest to travel. Last summer, Bairlein collected 10 wheatear hatchlings from nests in Norway and nine from Iceland. His team hand-raised the birds in artificial nests with the light and temperature each would have experienced in the wild.

Despite having access to the same amount of food, the hatchlings from Iceland gained an average of 7 to 8 grams, while those from Norway added about half that amount. "They must accumulate a certain level of fat for these long migrations," says Bairlein. The extra grams on the Icelandic birds "would enable a nonstop flight of at least 1200 kilometers."

The researchers also observed that the birds developed the migrant's nocturnal restlessness in the fall, coinciding with the peaking of their body mass. "They are somewhat like robots," Bairlein says. "They have a preprogrammed body mass index they must attain and a time of year they must depart."

Yet wheatears also have "a surprising amount of behavioral flexibility," he notes. From their Helgoland field research, he and his colleagues have discovered that wheatears can delay their journeys for a few days if the weather is too rough. They detour around storms, too. They also depart earlier from their resupply sites if there are too many predators (primarily raptors and cats) about, or if the feeding grounds are overcrowded with other wheatears.

That flexibility may save the species if, say, refueling stops vanish as a result of climate change or agricultural practices. Birds that have some ability to override their innate migratory programs and fly to another stop will have a better chance at survival, Bairlein says. But if the Sahara continues to increase in size, turning the birds' African destination into desert, the outcome is less certain. Already, other migratory songbirds are showing signs of decline, and the wheatears' fate may be similar. "That's what we're watching now," says Bairlein, "to see how rapidly this inherited, genetic system can respond to environmental change."

–VIRGINIA MORELL

Virginia Morell is a writer in Ashland, Oregon

NEWS

Follow the Footprints

An ancient tracking method goes high-tech to keep tabs on large, secretive animals

For endangered black rhinos, which roam in small pockets across southern and western Africa, radio collars have been the technology du jour. But in the 1990s, a wife-husband team tracking this endangered species decided collars just weren't the way to go.

"You'd be looking for an animal, and sometimes you'd lose the signal, and you wouldn't know if the collar had dropped off or if it was still on," says Zoe Jewell, who with her husband, Sky Alibhai, runs an organization based in Portugal called WildTrack. The pair would occasionally find rhinos whose efforts to remove the collars broke the radio transmitters and left the animals with deep lacerations in their necks. "It made us wonder how many rhinos were walking around with collars cutting into them, not working," Jewell says. In 2001, they reported that within a year of fitting 61 rhinos with radio collars, the equipment had failed for 73% of males and 44% of females.

The collars also occasionally created a threat to the researchers themselves. As aerial monitoring was too expensive for daily tracking, the couple used handheld receivers. When the receiver and transmitter are close together, the "beeps" can come so fast and furious that it's tough to locate the animals. "Quite often in wooded areas, the beeps would be all around you," says Jewell. "We had some very scary instances," she adds, when they discovered a rhino right behind them and had to scramble up a nearby tree.

The desire for a better—and safer—tracking method has since led Jewell, a veterinarian, and Alibhai, a zoologist, to develop the footprint identification technique (FIT). Dubbed WildTrack, FIT is a high-tech method for identifying individual animals by their footprints. There's growing interest in WildTrack by researchers looking for innovative ways to census and study animal populations, particularly for conservation purposes. But there's also skepticism in the community. "I would like to see a publication justifying some of these track-discrimination statistical approaches in a good peer-reviewed biostatistics journal," says K. Ullas Karanth, a wildlife biologist with the Wildlife Conservation Society in Bangalore, India. For other researchers, radio collars' advantages outweigh any disadvantages.

For the WildTrack duo, there's room for both technologies. "We're not against radio collaring per se," says Alibhai. "It's the way it was being used." For example, in Zimbabwe's parks, managers sought to collar every rhino within the protected area's boundaries. Aside from the potential harm to the animals and the close calls between researchers and rhinos, the immobilization and capture required to put the collars on the rhinos compromised female fertility, according to Alibhai.

Between 1994 and 1997, he and Jewell measured annual live births and conception, birthing, and fertility rates in 46 female black rhinos that were immobilized a total of 113 times during that period. They found that the more often a female was immobilized, the greater the negative effect on each of these fertility measures.

The couple had difficulty getting their results published, and when their research article did come out in the March 2001 *Journal of Zoology*, their collar-using colleagues

Making an impression. Rhinos can be hard to find in the wild, but their tracks tell the animals' whereabouts.

CREDITS (TOP TO BOTTOM): KARL H. SWITAK/PHOTO RESEARCHERS; ZOE JEWELL AND SKY ALIBHAI/WILDTRACK

seemed loath to accept the results. "We were almost blacklisted," Jewell recalls.

They realized, however, that an alternative to radio collars lay, literally, just under their feet. Impressed by the ability of local rangers to interpret and follow individual rhino tracks, Jewell and Alibhai began toying with the idea of adapting footprint tracking for their needs. But they quickly became stumped. "We knew what we wanted to do, but we didn't have the tools to do it," Jewell says. Their early efforts, starting around 1992, involved tracing prints onto transparent sheets placed on the ground, but this technique was inevitably fraught with human error. After a full season of tracing, they found that their measurements weren't reliable enough to identify individuals.

Then came the digital revolution. With digital cameras and scanners, the researchers added more objectivity to the recording process. To have confidence that they could really tell one individual's track from another's, they used a well-documented group of 15 wild black rhinos in Zimbabwe to determine features that consistently differ from one rhino to the next.

A rhino's footprint looks vaguely like a three-leaf clover. The main orienting points are the three toes and the heel. Rhinos also have lines on the heel, a bit like human fingerprints, which can be picked up in good tracks. Jewell, Alibhai, and Peter Law, a mathematician and independent scientist, developed a computer program to identify individual rhinos by their tracks.

Always using footprints from the left hind foot, the team put 13 "landscape" marks at key points of the print, such as at the top of the toes, between the toes, and at the lowest point on the heel. They then took 77 measurements for each track, consisting of both lengths and angles within the footprint. Of these 77, 30 were able to discriminate individual tracks. In a May 2001 *Journal of Zoology* article, the team reported that FIT accurately identified black rhinos up to 95% of the time.

Jewell and Alibhai trained antipoaching scouts in Zimbabwe to take photos of rhino tracks. With these images, the researchers were able to determine which animal had moved where. They learned that the range of a black rhino seems to vary greatly between individuals, from 30 to 300 square kilometers. Knowing the range of a single rhino or a single group can give managers a sense of how extensively their antipoaching units should patrol, Jewell points out.

Other researchers are now following in the couple's tracks. Patricia Medici, head of the Tapir Specialist Group in Brazil, is currently using WildTrack to study lowland populations of these piglike mammals in Brazil, focusing on a similar set of landscape mark measurements to distinguish one three-toed hind track from another.

White on white. By analyzing polar bear footprints, scientists expect to get a more reliable head count for these elusive animals.

She was lured by the relative frugality of FIT. Prior to using the tracking method, she had been doing radio telemetry on tapirs for 10 years, with collars and tracking gear costing more than $1000 per animal. "All the equipment is really expensive, and it requires a huge effort in the field all of the time," says Medici.

Graduate student Linda van Bommel of Wageningen University in the Netherlands has recently compared several methods for analyzing footprints. She evaluated prints from 30 wild and captive lions. FIT proved to be the most accurate and has the potential to work particularly well in South Africa, she says. The large carnivores there tend to follow roads, where passing vehicles kick up dust. Lions walking in that thin layer of dust "leave really perfect footprints," van Bommel explains. She's currently analyzing photographed footprints from nine South African lions.

WildTrack isn't for every researcher, every species, or every place. Leaf-littered forests, floodplains that are constantly wet, rocky terrain, and some other habitats are not conducive to tracking. And Alibhai says that footprints need to have complex features in order to distinguish one individual from another. The strategy thus might not work as well with species with single toes, such as zebras, or with hard-hoofed ungulates.

Some researchers are skeptical of WildTrack's utility. Although the footprints tell a researcher where an animal has been, they don't tell where it is at any given moment. Radio collaring gives scientists the ability to "follow [the animal's] movements and do 24-hour tracking," says Eric Dinerstein of the World Wildlife Fund in Washington, D.C. Thus, researchers can corner the animals at will and collect tissue samples to analyze DNA and do veterinary tests.

However, the cost savings and other advantages of FIT continue to bring in inquiries from scientists. So far, it's been adapted for lowland and Baird's tapirs, white and black rhinos, and Bengal tigers. Plans are in the works for Sumatran rhinos, leopards, giant sable antelopes, and dholes (Asiatic wild dogs). And just recently, the couple began a collaboration with Peter de Groot of Queen's University in Kingston, Canada.

De Groot wants to adapt FIT to analyze the snow tracks of polar bears. In partnership with local Inuits, who desire a say in the management of polar bear hunting, he had been looking at DNA from fecal samples to census the bears on Canada's King William Island. Combining the genetics and tracking would provide "two independent assessments that are noninjurious," he says, and give the Inuits and others a better understanding of the polar bear populations.

Because animals such as rhinos, tapirs, and polar bears can be hard to find, researchers benefit from using approaches that don't require animal sightings. "A lot of big vertebrates are tough to monitor, especially when they're rare or shy," says graduate student Jeremy Radachowsky of the University of Florida, Gainesville, who worked for the Wildlife Conservation Society studying Baird's tapirs in Guatemala and has used WildTrack for his studies. "It's an incredibly powerful method. It has the potential to change wildlife monitoring."

–KATHERINE UNGER

Former *Science* intern Katherine Unger is a staff writer at the Wildlife Society in Bethesda, Maryland.

PERSPECTIVE

Long-Distance Dispersal of Plants

Ran Nathan

Long-distance dispersal (LDD) of plants poses challenges to research because it involves rare events driven by complex and highly stochastic processes. The current surge of renewed interest in LDD, motivated by growing recognition of its critical importance for natural populations and communities and for humanity, promises an improved, quantitatively derived understanding of LDD. To gain deep insights into the patterns, mechanisms, causes, and consequences of LDD, we must look beyond the standard dispersal vectors and the mean trend of the distribution of dispersal distances. "Nonstandard" mechanisms such as extreme climatic events and generalized LDD vectors seem to hold the greatest explanatory power for the drastic deviations from the mean trend, deviations that make the nearly impossible LDD a reality.

Dispersal, defined as the unidirectional movement of an individual away from its place of birth (*1*, *2*), is a widespread phenomenon occurring in nearly all organisms. In plants, as in other sessile organisms, dispersal is mostly passive: Seeds or other diaspores (dispersal units) are transported away from the parent plant by vectors such as animals, wind, and water (*3*) (Fig. 1). In studying the ecology and evolution of processes such as dispersal, we usually focus our attention on the prevailing events, assuming that rare events are unimportant. Yet frequency and importance are not necessarily positively correlated. Here I highlight the rare, but disproportionately important, LDD events.

Dispersal is often portrayed in terms of the "dispersal kernel," the function that describes the probability of dispersal to different distances. The vast majority of seeds are typically dispersed short distances (*4*, *5*). The 2Dt dispersal kernel (*6*) (Fig. 2) is a "fat-tailed" distribution: LDD is more frequent than in a normal (Gaussian) or a negative exponential distribution with the same mean dispersal distance (in this case, 50 m), yet the probabilities of dispersal beyond a few hundred meters are very low. At a few hundred meters, LDD research encounters severe data limitation even for large, highly fecund trees, and data are even more restricted for most other plant species.

The fluctuating brown lines in Fig. 2 illustrate the enormous stochasticity associated with LDD. High stochasticity characterizes fecundity, the number of seeds produced, and seed-to-adult survival probability after dispersal. For a given dispersal kernel, fecundity determines how many dispersal events will actually occur, and post-dispersal survival determines what fraction of these events will lead to "effective dispersal" (i.e., successful establishment of reproductive individuals). In the hypothetical case shown in Fig. 2, the expected time for a single effective dispersal event to occur is longer than 100 billion years beyond 150 km. Nevertheless, an effective LDD event 415 km from the source, expected to occur once in almost 10^{13} years under the mean trend, may occur once in 10 years as a result of processes or events that "break the rules." Although the disparity between what is expected and what might appear seems absurd, we do have compelling evidence from many species that effective LDD events do occur far beyond the otherwise observed dispersal distances. This evidence comes from ecological studies (scales of several kilometers; see below) and biogeographical studies [e.g., multiple colonizations of remote islands such as Hawaii (*7*) and intercontinental disjunctions across the Atlantic Ocean (*8*)].

The rarity and stochasticity of LDD entail two fundamental difficulties in research: how to reliably quantify the tail of the dispersal kernel (i.e., the frequency and spatial extent of LDD events) (*5*, *9*), and how to construe the patterns, mechanisms, causes, and consequences of LDD, even if "perfect" data were available. These two difficulties and the immense variation in the spatial and temporal scales of LDD among individuals, populations, species, communities, and ecosystems (*2*, *10*) give rise to a third fundamental difficulty: how to define LDD. Indeed, any threshold distance used for identifying LDD is inherently arbitrary and case specific. Two major LDD definitions are most common (*11*): (i) an absolute threshold distance that may correspond to key biological and physical features [e.g., a distance of 250 m among patches in an experimentally fragmented forest (*12*)] and (ii) a relative threshold based on some percentile at the tail of the dispersal kernel [e.g., a fraction of 1% of all dispersed seeds having a mean dispersal distance of 500 m (*13*)].

Despite these difficulties, many researchers have become motivated by the recently renewed recognition of LDD's disproportionate importance (*1*, *2*, *5*, *10*, *11*). Classical studies have emphasized LDD's importance for colonization of oceanic islands (Fig. 1) and other remote habitats (*7*, *14*). More recent studies have shown that long jumps available through rare LDD events are much more influential than the numerous small steps available through local dispersal in determining the spread of invasive species or range expansion of native species after climatic range shifts (*6*, *15*). Rare LDD events also provide the essential link between habitat fragments (*12*) and facilitate species coexistence—for example, by enabling competitively inferior species to persist alongside competitively superior species through greater LDD capacity (*16*). In fact, LDD can facilitate coexistence even without such tradeoff between LDD capacity and competitive dominance (*13*). Indeed, LDD may also be selectively disadvantageous, as it reduces the ability to exclude competitors from occupied patches and to quickly exploit resources in newly available patches (*17*). Yet over-

Fig. 1. (**A**) *Epomophorus* sp. carrying a fig in Kenya. Large mammals, birds, and bats are traditionally considered LDD vectors. (**B**) LDD can also be mediated by abiotic factors such as strong storms, floods, and ocean currents that enabled plants such as sea sandwort (*Honckenya peploides*; front and back) and lyme grass (*Leymus arenarius*; middle) to colonize Surtsey, a volcanic island erupted from the ocean 33 km south of Iceland and 4.8 km from the nearest island seen in the background. LDD can also be mediated by "nonstandard" vectors. For example, seeds of five plant species with no known adaptation for water dispersal were drifted ashore attached to a "mermaid's purse" (inset), the egg capsule of the common skate (*Raja batis*). [Photographers: (A) Merlin D. Tuttle, Bat Conservation International; (B) Borgþór Magnússon and Sturla Fridriksson (inset), *Surtsey Research Progress Report* **6**, 25 (1972)]

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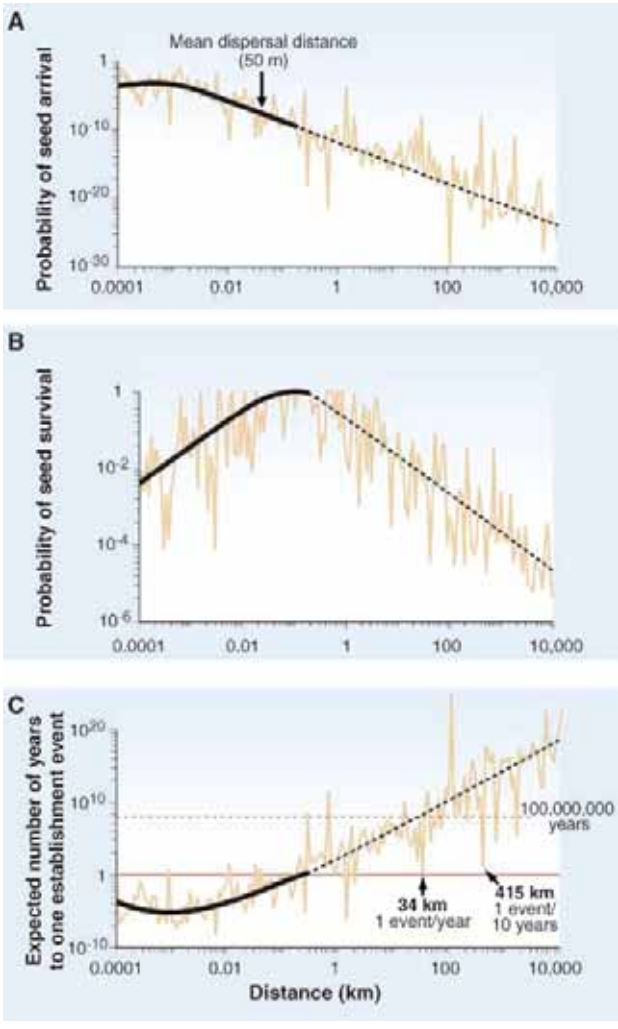

Fig. 2. Multiple temporal and spatial scales associated with LDD of seeds. The transition from bold to dashed lines at 0.2 km indicates the range of distances over which dispersal (and perhaps also survival) becomes practically unquantifiable by current methods; the fluctuating brown lines represent the stochastic deviations from the mean trend. (**A**) 2Dt dispersal kernel [see text; shape = 1.01, scale = 1050 m^2, mean dispersal distance = 50 m: values corresponding to the best LDD performance among tree species analyzed by (*6*)]. (**B**) Seed-to-adult survival kernel, combining a phase of increase [negative exponential mortality; survival = $1 - \exp(-0.04x)$] up to 200 m, and a phase of decrease (inverse power law; survival = $200x^{-1}$) farther away. The increasing survival phase follows the escape hypothesis, which postulates higher density-dependent predation and competition near the parent tree; the decreasing survival phase follows general spatial autocorrelation phenomena in which environmental conditions tend to be less similar, hence less suitable, farther away. (**C**) The expected number of years for establishment of one individual, calculated as the inverse of the product of dispersal (A) and survival (B) probabilities and the source strength defined as a population of 10^6 individuals, each with an annual fecundity of 10^4 seeds. LDD events over much of the range are extremely unlikely. Yet, as exemplified by the two cases indicated by arrows (C), large stochastic deviations generated by extreme events or generalized LDD vectors (see text) may raise the probability of LDD to levels that may be sufficient for LDD to be realized and detected even by contemporary ecological and genetic methods.

all, as we accumulate data and refine our models, we usually reinforce Darwin's (*14*) contention that across species and over time, LDD is a widespread phenomenon of great importance (*5*, *8*, *10*, *11*). In the following, I outline the recent achievements and the further research needed to quantify and understand the patterns, mechanisms, causes (evolution), and consequences of LDD.

Patterns of LDD: Pursue Better Data or Better Analyze Obtainable Data?

A variety of new methods provide better data for LDD of plant diaspores (*5*, *9*). The great potential ascribed to genetic methods to unveil LDD of seeds is beginning to show (*18*), but such studies are still uncommon and are limited in their spatial scale to a few hundred meters or less. Studies estimating effective dispersal kernels from genetic material taken from seedlings have been able to detect effective LDD events at scales of several kilometers (*19*). Genetic methods have also been used to assess the relative contributions of dispersal to gene flow by pollen versus by seed. Although the only way to colonize a distant location is through LDD by seeds, a common generalization asserts that LDD by pollen occurs over much larger spatial scales and hence is more important for interpopulation genetic structure. Yet some recent studies [e.g., (*19*)] of effective seed versus pollen LDD revealed that the opposite is true.

The above studies illustrate important attempts to provide more accurate data for depicting LDD. However, the hierarchical Bayesian (HB) approach better exploits existing data to infer the fundamental relationships that constitute the complex processes (see below) that give rise to LDD events (*20*). More accurate information is still necessary to guide HB models of LDD, so efforts to pursue better data complement attempts to develop better methods to analyze obtainable data.

Mechanisms of LDD: Should We Look Beyond "Standard" Dispersal?

LDD has long been considered to be "chance dispersal" (*7*), but for every passively dispersed diaspore there must be some vector, hence some mechanism, that takes it from one place to another. The past 5 years have seen active research into the mechanisms of LDD, and we now better understand the factors affecting LDD across plant populations and species. For example, the recognition that prolonged turbulent updrafts are critically important for LDD of forest tree seeds by wind (*21*) allows prediction of LDD from input data on wind attributes, seed terminal velocity (falling rate in still air), and height of seed release. A better understanding of the mechanisms also opens new directions for studying LDD evolution. For example, seed abscission is a plant-controlled trait that is likely to have much greater effect on LDD than seed terminal velocity, a property long considered to be the major determinant of dispersal capacity of wind-dispersed species (*22*).

An emerging generalization in LDD research asserts that morphological adaptations of the diaspore, typically used to identify the "standard" dispersal vector, determine short-distance dispersal but often do not constitute the main mechanism responsible for LDD (*23*, *24*). A single plant species may be dispersed by multiple dispersal vectors, including vectors that have traditionally been considered efficient for LDD (e.g., wind, water, birds, bats, and large mammals; Fig. 1), even in the absence of specific adaptations for each (*24*–*26*). Total dispersal kernels, which incorporate the contribution of multiple dispersal vectors, are important to consider when different vectors act differently at different scales (*24*). Large herbivores, for example, disperse viable seeds of multiple plant species with diverse diaspore types over large distances (*25*, *26*). The same holds for dispersal by strong updrafts (*21*) and streams (*27*). Such generalized LDD vectors, which routinely disperse a variety of species and diaspore types over long distances, may drastically increase the probability of LDD relative to the "standard" dispersal vectors.

Extreme events are another potential explanation for LDD. A longstanding hypothesis proposes that tropical cyclones can disperse diverse life forms over very long distances (*28*), but quantitative investigation of such extreme events has not yet been conducted. Extreme events include weather events of unusual power that occur irregularly yet are not necessarily rare; for example, hurricanes, typhoons, and tornadoes occur rather frequently in ecological time scales. Intentional or accidental human-mediated LDD—probably not a new phenomenon (*28*)—can also be considered an extreme event, although, given

the current all-inclusive anthropogenic impact on Earth, people might be better considered a generalized LDD vector. Human-mediated LDD is almost certainly now the single most important mechanism of LDD of plants and animals.

Causes of LDD: What Drives LDD Evolution?

Theoretical studies have been instrumental in elucidating the general causes and conditions for the evolution of dispersal, emphasizing the role of spatial and temporal heterogeneity, kin competition, and inbreeding depression (*1*, *10*). Recent theoretical studies have shown that dispersal kernels of different shapes with correspondingly different LDD levels are favored under different sets of conditions (*10*, *29*). Thus, LDD in particular, and not only dispersal in general, may have adaptive value and hence can be favored by natural selection. Furthermore, it has been shown that spatially explicit models incorporating realistic dispersal kernels can elucidate ecological and evolutionary dynamics such as the interplay between relative abundance and the strength of selection for LDD (*29*) and between local population dynamics and LDD (*13*) that cannot be explained by models that treat dispersal simplistically.

At present, given advances in theoretical techniques and computing power, understanding of LDD evolution is limited mostly by the dearth of empirical data on the costs and benefits of LDD (*10*, *29*), and even how it occurs. The key questions about LDD evolution are still fairly fundamental: Which traits and conditions promote (or deter) LDD de facto? What are their tradeoffs with short-distance dispersal and with other fitness components? To what extent can they be controlled, directly or indirectly, by the plant? If generalized LDD vectors and extreme events determine LDD irrespective of the diaspore morphology, it is likely that the diaspore morphology is molded by natural selection for the benefits of short-distance dispersal by "standard" vectors, regardless of the benefits of LDD. The question is then whether LDD is a process over which plants have little or no control, or whether it influences the evolution of traits other than the diaspore morphology (e.g., palatability to large herbivores). LDD evolution studies should be redirected to the mechanisms that actually drive LDD.

Consequences of LDD: How Does LDD Affect Populations and Communities?

LDD can clearly play a major role in shaping a variety of ecological and evolutionary processes. However, this role has been thoroughly investigated only for population spread (invasive species and postglacial expansion), for which the importance of the dispersal kernel shape and the actual level of LDD have been forcefully demonstrated (*6*, *10*, *15*). To explore the consequences of LDD for other population-level processes and for all metapopulation-, community-, metacommunity-, and ecosystem-level processes, our simplistic assumptions about dispersal should be relaxed and LDD should be incorporated realistically (*10*, *30*).

A prime example is Hubbell's (*31*) unified neutral theory, which controversially assumes that all individuals of all species are equivalent and assigns a critical role for dispersal limitation (i.e., very low LDD) in shaping communities. This theory folds all dispersal dynamics into a single parameter m, the probability that an unoccupied site will be colonized by an immigrant. Recent theoretical studies that did incorporate realistic dispersal kernels in spatial models show that the scale of dispersal (or the level of LDD) can strongly influence community patterns in both neutral and non-neutral models (*32*). It should be emphasized, however, that all these studies have applied the same dispersal kernel for all species; incorporating between-species variation in dispersal is likely to provide new insights into the study of community dynamics. The key problem is that we often lack knowledge of the dispersal kernel of even a single species in a community.

Synthesis

LDD research is severely constrained by data limitations. Although our progress in recent years is encouraging, we must seek further improvements in our ability to estimate the probabilities of LDD. Estimating the "mean trend" of dispersal is challenging yet important; however, estimating the deviations from the mean trend is even more challenging and even more important (Fig. 2). Practically speaking, it will always be impossible to predict a single LDD event or to estimate the probability of seed arrival to a specific location far away from the seed source. Yet reliable estimates of the distribution of the deviations are within reach, and deeper understanding of the underlying mechanisms is the best way to accomplish this goal.

To better understand LDD, we should take several steps. First, we need to develop better methods to quantify LDD and to better integrate existing methods, including genetic and ecological techniques, telemetry tracking of diaspores and dispersers, and models of different kinds. We should also further develop the statistical methods to extract information from more readily obtainable data. Second, we need to consider dispersal by multiple vectors, probably the rule rather than the exception (*24*), and accommodate differences in the spatial and temporal scales within which different vectors act. Third, we need to develop mechanistic models of LDD by animals and by abiotic factors other than wind, to use spatially explicit models with stochasticity and realistic dispersal kernels in all aspects of LDD research, and to test them rigorously through field manipulations or observations. Fourth, to better investigate the role of landscape structure, generalized LDD vectors, and extreme events, our research must encompass the multiple spatial and temporal scales over which LDD operates [e.g., (*33*)]. This calls for closer integration among ecologists, environmental scientists, population geneticists, paleontologists, and biogeographers. Finally, we should not isolate dispersal from the general context. Dispersal studies should be coupled with investigations of pre- and postdispersal factors affecting fecundity, population size, survival, and establishment.

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PERSPECTIVE

From Individual Dispersal to Species Ranges: Perspectives for a Changing World

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Dispersal is often risky to the individual, yet the long-term survival of populations depends on having a sufficient number of individuals that move, find each other, and locate suitable breeding habitats. This tension has consequences that rarely meet our conservation or management goals. This is particularly true in changing environments, which makes the study of dispersal urgently topical in a world plagued with habitat loss, climate change, and species introductions. Despite the difficulty of tracking mobile individuals over potentially vast ranges, recent research has revealed a multitude of ways in which dispersal evolution can either constrain, or accelerate, species' responses to environmental changes.

From sticky seeds to efficient flight machinery with complex navigation systems, animals and plants have evolved an impressive variety of dispersal mechanisms. Through the simple act of moving individuals from one area to another, dispersal has important ecological and evolutionary consequences (*1*, *2*), including the ability of species to change or expand their ranges (*3*). The distribution of species we observe today reflects a long history of alternating episodes of dispersal and isolation. Fluctuations in sea level that opened and closed land corridors, the splitting of continents, and the rising of mountain ranges and islands have all left their mark on the whereabouts of extant species. Nowadays, humans are creating new processes that isolate, connect, and shift landscapes at a much higher speed: Anthropogenic habitat fragmentation, transport of invasive species, and climate change are among them. How will species ranges react to them? How will their dispersal behavior change? Are physical barriers and open corridors all there is to explain species distributions?

To predict whether species can shift to new areas requires, on the one hand, understanding the colonization process at the expanding edge of the species range. On the other hand, the possible range contraction where habitat is deteriorating depends crucially on whether individuals simply leave poor-quality habitat or attempt to stay. Local adaptation to changing conditions is also possible, yet strong dispersal can swamp local genetic change and so prevent adaptation from happening (*4*). Current anthropogenic environmental changes make the study of the evolution of dispersal a requirement for predictive ecology (*5*).

Dispersal is an important determinant of gene spread and is thus subject to strong natural selection (*1*, *2*, *6*). Even if dispersal is risky, it can evolve to avoid the detrimental effects of crowding and competing with kin (*6*). An evolved willingness of individuals to move about is an obvious prerequisite for the spread of a species to different parts of its fundamental niche. But how well do organisms actually achieve this? Evolutionary ecologists are increasingly aware that not only limitations of cognitive abilities, but also selective pressures themselves, can cause severe constraints on the habitat use of a given species. In other words, intrinsic species properties, rather than just limitations imposed by the landscape, can have profound effects on the ability of a species to colonize new areas (*3*). Unraveling these mechanisms is important if we are to understand the evolution of species ranges and how they respond to environmental change.

Adaptation Does Not Predict Optimal Space Use

The ability to distinguish between suitable and less suitable habitats is an obvious first limitation to colonization of new areas. Dispersal and habitat settlement cues are often based on "rules of thumb" that can be breathtakingly simple. This can lead to ecological traps, where environmental change dissociates habitat quality from the cues and causes individuals to prefer suboptimal habitats (*7*). For example, dragonflies have been observed to patrol asphalt roads instead of rivers, which results from their use of polarized light as a cue for still water and can also lead to a strong preference for landing on oil (*8*). Another simplistic cue is conspecific attraction, where individuals use the presence of others as an indicator of good habitat: Playbacks of song attract young bobolinks *Dolichonyx oryzivorus* to settle in habitats of random quality (*9*). If individuals simply copy others' choices, one can envisage a self-reinforcing and intrinsically random component to habitat use.

But attraction to conspecifics can be more than just an easy cue indicating suitable habitat. In many species of animals, individuals directly benefit from living in groups; philopatry (i.e., staying in the natal patch) can be selected for, particularly if local habitats are worth clinging to

Fig. 1. Dispersing individuals are a nonrandom subset of the population. (**Left**) Dispersing females of the Glanville fritillary, *Melitaea cinxia*, have a higher flight metabolic rate and are more fecund than sedentary ones. [photograph: Anne Holma] (**Right**) In Siberian jays, *Perisoreus infaustus*, the subordinate individuals disperse, whereas the heavier and more dominant remain in their natal territories. [photograph: Hannu Siitonen] Differences in selective pressures on dispersal may have profound consequences for the stability of newly founded populations and, consequently, for the ability of the species to spread and react to environmental change.

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(*10*). The home advantage is particularly clear in social species, in which cooperative behaviors may enhance the value of home. When the natal patch has particular value, staying at home is often the privilege of the dominant offspring, and subordinates are forced to leave [e.g., the Siberian jay, *Perisoreus infaustus* (Fig. 1) (*11*)].

Regardless of the degree of sociality, the short-term fitness advantage of staying at home can contrast with the longer term ability of a species to colonize available patches (*12*). Thus, although some dispersal is always selected for, there is no guarantee that the evolving dispersal rate leads to the best possible persistence of a species. Similarly, improved cognitive abilities, while advantageous to the individual, do not necessarily lead to more efficient use of space. Computer simulations show that "blind" dispersal in a random direction can connect populations better than behaviors that allow habitat assessment to take place (*13*).

The consequences of such theoretical results are profound: It is even possible that a metapopulation (a set of populations connected by dispersal) evolves itself toward extinction. This can happen if dispersal becomes riskier, for example, because suitable patches have become scarce. In this case, individuals are selected to avoid the dangerous dispersal phase, yet the metapopulation cannot persist in the absence of a continuous influx of migrants (*14*). Predicting the direction of the evolutionary response is challenging, however. The failure of most dispersers in a scenario of risky dispersal will vacate much of the landscape, which can paradoxically enhance the fitness rewards for those lucky dispersers that survive. Under suitable conditions, this favors higher dispersal, potentially rescuing entire metapopulations—a scenario that has been shown to arise with parameters from populations of checkerspot butterflies, such as the Glanville fritillary, *Melitaea cinxia* (Fig. 1) (*15*).

Nevertheless, philopatry has its bright side too, which becomes visible when a species faces a different kind of change in its environment: the dissociation of cues and habitat quality that characterizes ecological traps. Philopatry dictates that wherever the production of young has been most recently successful is precisely where most individuals prefer to reside, and this may allow the species to track changing environments much faster than a genetic change in habitat choice cues would allow (*16*). Such population-level benefit of philopatry of course requires that at least some individuals have found the currently best habitats. This is ultimately dependent on dispersal, which, being the flipside of philopatry, highlights the complexity of population consequences of rules of movement.

Near the margin of a species' distribution, populations are precariously balanced on the edge between persistence and extinction, and ranges can easily become "pinned" to a fixed area by poor performance in marginal habitats or low densities (*17*). Such restrictions are important in a world in which shifts in climate will pose a dramatic challenge to species persistence. Dispersal is a trait that often shows considerable phenotypic plasticity, responding to local density either positively or negatively (*1*). Cases where it occurs as a response to local crowding are highly relevant when predicting responses to habitat loss and shifting climates. Such shifts imply that locally declining populations could have better prospects elsewhere. Because the initial decline decreases local crowding, the result may be a decline in dispersal that prevents the population from finding the improved habitats elsewhere (*18*).

...Yet Space Use Can Accelerate Adaptation

Dispersing individuals take their genes with them. Thus, any genetic trait that influences the tendency or ability to disperse can easily cause spatial differentiation in the gene pool. Finding new habitats is an essential prerequisite for breeding in them, and thus, dispersal is a good example of a trait that can experience strong selection in marginal areas. Indeed, in Glanville fritillary (*Melitaea cinxia*) metapopulations, recently colonized patches are mainly composed of individuals with higher flight ability and fecundity than those found in old patches (*19*). Interestingly, allelic variation in a single gene can contribute significantly to this variation (*20*). Other species instead show trade-offs between fecundity and dispersal (*21*). Recent theoretical work shows that such differences can determine the stability of the species' range boundary and, consequently, play a decisive role in whether the species will expand its range (*22*).

Several recent examples suggest that evolution can be both fast and significant for the ability of a species to colonize new areas. The evolution of more dispersive individuals near species borders clearly creates a positive feedback that has potential to accelerate the expansion of a species. Marginal populations of bush crickets (*Conocephalus* and *Metrioptera* spp.) expanding their ranges through the United Kingdom showed increased frequencies of long-winged dispersive individuals, which indicates evolutionary change (*23*). In an unfortunately spectacular example, cane toads, *Bufo marinus*, introduced into Australia are rapidly invading the continent, helped by fast adaptation that produces longer-legged individuals and enhances dispersal at the invasion front (*24*). Nevertheless, recent evidence suggests that invasions are not simple uninhibited traveling waves, but follow regulatory patterns analogous to the long-studied forms of density dependence within stationary populations (*25*). It will be interesting to examine the causes behind this apparent regulation and to find if different dynamics at range margins can be predicted depending on the determinants of dispersal behavior.

Future Perspectives

The above examples show great diversity in the potential responses of species to environmental change, determined by the constraints and selective pressures on dispersal. However, to get beyond a list of representative examples, we still need a comprehensive framework that links theoretical advances to empirical case studies. Without an understanding of why, for example, individuals of some species disperse from locally crowded habitats and others do not, we cannot make solid predictions on how species will react to new conditions.

There are empirical challenges too. It is not surprising that local population regulation is much better studied than the regulation of invasion fronts (*25*) or other dynamic aspects of dispersal: Tracking mobile individuals over potentially large distances presents obvious difficulties. Advanced techniques, such as stable isotope analysis or molecular methods, provide much insight, but to be truly useful, the results always need an ecological context (*20*, *26*). An additional challenge is to understand the failure of some species to establish in new areas. The absence of populations requires explanation as much as their presence, and comparisons between populations or closely related species that differ in their ability to colonize new areas promise new insights into the question (*23*, *27*). But to identify clearly the causal mechanisms behind dispersal, experiments will provide the most solid answers. This may seem challenging, yet there already exist examples of experiments conducted either in the field (*9*, *10*, *28*) or in artificial environments (*29*), demonstrating that one can move beyond correlational approaches for examining the causes of dispersal. Further work on model organisms with sufficiently short generation times promises an exciting way to test evolutionary responses of dispersal to environmental change.

We are surrounded by cases of unwelcome species introductions, as well as numerous failures of populations to persist in fragmented, human-disturbed habitats. This provides researchers with a unique large-scale experiment and valuable source of data to understand the evolution of dispersal. Ironically, such data are precisely what we need to better predict species reactions to change and to prevent the loss of biodiversity in an era of global warming, habitat loss, and invasive species.

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PERSPECTIVE

Conflicting Evidence About Long-Distance Animal Navigation

Thomas Alerstam

Because of conflicting evidence about several fundamental issues, long-distance animal navigation has yet to be satisfactorily explained. Among the unsolved problems are the nature of genetic spatial control of migration and the relationships between celestial and magnetic compass mechanisms and between different map-related cues in orientation and homing, respectively. In addition, navigation is expected to differ between animal groups depending on sensory capabilities and ecological conditions. Evaluations based on modern long-term tracking techniques of the geometry of migration routes and individual migration history, combined with behavioral experiments and exploration of the sensory and genetic mechanisms, will be crucial for understanding the spatial principles that guide animals on their global journeys.

Migration emerges and becomes suppressed among birds, fish, insects, sea turtles, bats, and sea mammals with apparent evolutionary ease. It has evolved numerous times without important phylogenetic constraints; closely related species or populations are often nonmigratory. Furthermore, postglacial changes in travel patterns indicate a high degree of evolutionary flexibility in migration traits. Thus, as noted almost a century ago by the American ornithologist Joseph Grinnell (*1*), the evidence implies that migratory adaptations are rather simple extensions of capabilities that animals use for their everyday local life and movements. Yet after longstanding and intensive migration research, we are still far from a fundamental understanding of animal navigation, and the emerging picture is complex and intricate.

Linnaeus, in his treatise *Migrationes Avium* (1757), appealed for field observations from all over the world that would reveal birds' migration routes. Over the next two centuries, banding of birds and fish produced a wealth of information, and displacement experiments revealed impressive homing performances. Orientation cage experiments proved to be powerful for discovering and exploring celestial as well as magnetic compass mechanisms. The map sense that is required in addition to a compass sense to explain the homing capabilities of animals attracted increased attention (*2*, *3*). Animal tracking studies have benefited during the recent decades from increasingly sophisticated techniques, such as radar registration, satellite-based radio telemetry, and electronic geolocation and data storage tags. However, current research is characterized by conflicting evidence and interpretations about several fundamental questions (Table 1).

Until recently, it was believed that juvenile birds (among species traveling solitarily) on their first journeys relied solely on an endogenous spatiotemporal program, defining the journey in terms of direction and distance along one or a few main legs (Table 1B). Adding successive travel steps for migrants guided by such a simple inherent clock-and-compass program, with some variation between each step, will lead to a geographic spread of a population of migrants that increases with distance as a parabola along the migratory axis. Ringing recovery distributions of some bird species fit nicely with this predicted pattern (*4*).

However, migration patterns converging toward narrowly defined species-specific passage or wintering areas can hardly be the result of such simple endogenous control (*5*, *6*) (Fig. 1). Rather, these patterns indicate that migrants use external cues, such as geomagnetic coordinates (e.g., magnetic field strength and inclination). Thrush nightingales (*Luscinia luscinia*) that were experimentally moved in geomagnetic space (while retained geographically in Sweden) to their target stopover area in Egypt increased their fuel deposition as expected when preparing to cross the Sahara desert (*7*). The best evidence for geomagnetic coordinates as regional markers comes from experiments with hatchling loggerhead sea turtles (*Caretta caretta*) (*8*). The turtles changed their orientation in relation to geomagnetic position so as to remain along the migration route at the North Atlantic gyre. However, inherent magnetic map guidance is not without complications. The current difference in magnetic coordinates between northern Florida and the northeastern gyre will be obliterated in less than fifty years because of differential geomagnetic secular changes at these two places. Geomagnetic coordinates at the thrush nightingale's target area, in 100 to 150 years time, will have changed to those that today are found at the target area of the blackcap (*Sylvia atricapilla*) (Fig. 1). How is it possible for evolutionary change in the animals' genetic migration program to keep pace with such secular changes in the Earth's magnetic field (Table 1H)?

Homing may be an important element in migration of experienced animals that return to favorable sites visited earlier, such as breeding and wintering destinations and goal areas along the route (Table 1C). If the geomagnetic field provides information for migration control, it may also be an important basis for the map sense used by homing animals, an idea that has been considered several times since the end of the 19th century (*3*). Recent experiments in which animals have been displaced in geomagnetic (but not geographic) space have provided support for this possibility among salamanders, spiny lobsters, and sea turtles (*9*–*11*). However, the hypothesis of homing based on a magnetic map sense is controversial. Supporting experiments have demonstrated homing responses to geomagnetic north-south displacements but not yet to east-west displacements, where the differential changes in geomagnetic parameters are more critical. It also remains to be shown whether geomagnetic gradients allowing unambiguous navigation are actually available within the local natural homing ranges of the experimental animals. An even more serious difficulty for the magnetic navigation hypothesis is the failure reported in several experimental attempts to disrupt homing success by attaching magnets to the animals (*3*). Sea turtles, petrels, and albatrosses equipped with disturbance magnets and recorded by satellite tracking show oceanic navigation performance similar to that of control individuals (*12*–*15*).

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These results imply that magnetic cues are not essential for successful homing if alternative navigation cues are available. A possible alternative homing mechanism is olfactory navigation. Seabirds have an excellent sense of smell and local emissions of scents released by phytoplankton may create an odor landscape that seabirds use for their navigation and homing (*16*). Green turtles (*Chelonia mydas*) that were displaced away from Ascension Island and recorded by satellite tracking relocated the island most efficiently from the downwind direction, suggesting that they used wind- or water-borne cues for their homing (*17*, *18*). For homing pigeons, olfactory navigation remains theoretically possible on the basis of observed large-scale gradients of atmospheric chemical substances (*19*).

For birds, the intercalibration between different compass mechanisms has been studied in cue-conflict cage experiments [in which birds are exposed to both celestial and magnetic cues, with one or both of these cues manipulated such that they give conflicting compass information (Table 1D)]. A consensus seemed to emerge that, for nocturnal migrants, twilight and star compasses are recalibrated to the geomagnetic field during the migratory period [whereas during the juvenile premigratory period celestial rotation constitutes the calibration basis for the magnetic compass (*3*)]. However, this view was recently disputed by an elegant experiment in which *Catharus* thrushes were exposed to a deflected magnetic field during twilight and tracked by radio telemetry on their subsequent night flights (*20*). The outcome indicated, contrary to expectation, that the thrushes recalibrated their magnetic compass in relation to the twilight cues and then relied on their (miscalibrated) magnetic compass for the nocturnal flight, apparently ignoring stellar cues. The experimental birds changed to normal orientation again on succeeding nights when they had recalibrated their magnetic compass back to normal. A recent review of cue-conflict experiments proposes that the birds' view of the twilight sky near the horizon may be decisive for the calibration rank between magnetic and celestial cues (*21*). There may also exist important differences between species in compass mechanisms and their hierarchical organization. New studies, particularly of birds under natural free-flying conditions, are now needed for a reevaluation of the compass calibration issue.

The geometry of migration trajectories may indicate whether animals travel on constant compass courses over longer distances and, if so, which compasses are dominant (Table 1E). Tracking by ground-based radio telemetry suggested that a *Catharus* thrush oriented at a constant angle in relation to the sunset azimuth during six successive nocturnal flights over a total distance of 1500 km (*20*). Radar studies have indicated that shorebirds follow flight trajectories similar to great circles on long-distance flights across the Arctic Ocean at Siberia and arctic North America. Sun compass orientation, without correction for the longitudinal shift in local time, provides a possible mechanism for great circle orientation at polar latitudes (*22*). However, great circle routes are not universal. There are also several cases where bird migration routes conform most closely with a constant compass course. Trajectories of raptors in both the Old and New World were generally more consistent with constant geographic courses (implying dominant orientation by celestial cues) than constant geomagnetic courses (*23*). Further and closer analyses of the geometry of migration routes seem to be a promising avenue for the future.

Table 1.

Examples of unsolved problems and questions with conflicting evidence and interpretations in the field of animal navigation

A	How can the existence of complex mechanisms in animal navigation and migration be reconciled with short evolutionary transition steps between nonmigratory and migratory status?
B	How important are external signals from map-related factors in the spatiotemporal control of migration of naïve animals?
C	How important are geomagnetic, olfactory, and other map-related cues in the homing of different animals?
D	What is the relative importance of the individuals' multiple compass mechanisms based on celestial and magnetic cues, and how are the different compasses calibrated in relation to each other?
E	How do orientation and navigation mechanisms influence the geometry of migration routes, and to what extent do migrants travel on constant compass courses or along great circles?
F	How does learning during early migration episodes influence subsequent journeys with respect to, e.g., route and goal area fidelity, and how does navigation develop with age?
G	How do specific and total demands on brain capacity differ between migrants and residents?
H	What are the possibilities and constraints for rapid evolution of new migration routes?

Fig. 1. Ringing recoveries in the eastern Mediterranean region of trans-Saharan passerine migrants (both adults and juveniles). Triangles indicate endpoints. Birds with similar general habitat requirements from Fennoscandian breeding grounds show species-specific autumn passage and stopover areas, as exemplified for the thrush nightingale (*Luscinia luscinia*) (yellow) and the blackcap (*Sylvia atricapilla*) (red). Responses by the migrants to external navigational signposts or triggers seem to be required for locating such well-defined target areas. Thin lines connect ringing and recovery locations but do not show the routes followed by the birds between these two points. [Image based on (*6*)]

The exploration of long-distance migration has entered a new era with a rapid growth of individual-based tracking data of increasing spatial resolution and prolonged coverage during multiple years and journeys. This will be crucial to understand how migration is organized into phases of dispersal, exploration, compass orientation, and homing, and how learning during one journey affects succeeding journeys (Table 1F). Individuals of North Sea plaice (*Pleuronectes platessa*) showed a high degree of fidelity to previous routes and destinations on their 250-km-long journeys between feeding grounds and spawning areas, sometimes within 20 km of their positions in previous seasons (*24*). Bluefin tuna (*Thunnus thynnus*) were also faithful to their spawning sites but with migration patterns developing with age in a complex way, as recorded for individuals continuously tracked during 3 to 5 years (*25*). Migration patterns of gray-headed albatrosses (*Thalassarche chrystostoma*) tended to fall into different categories with individuals in the most common category undertaking complete circumnavigations around the Antarctic continent (*26*). Adult ospreys (*Pandion haliaetus*) revisited one or a few individual intermediary goal areas during their repeated journeys between north Europe and West Africa (*27*). Between these goal regions route fidelity was low, and flight paths by the same individual often ran several hundred kilometers apart on different journeys, far beyond visual landmark contact (Fig. 2). It is likely that the ospreys could find the next goal region by regional map-based navigation, possibly in combination with path integration.

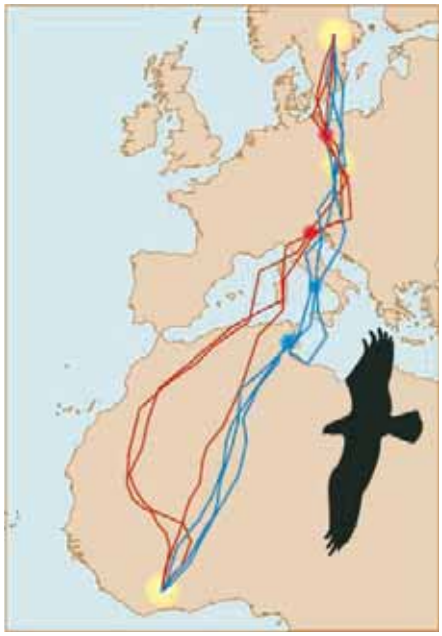

Fig. 2. Routes followed by the same individual osprey (*Pandion haliaetus*) during three successive autumn (blue) and spring (red) migration journeys, as recorded by satellite tracking. The bird showed fidelity not only to its breeding and wintering site but also to an intermediary stopover site in Europe that was visited on all journeys (yellow). Additional possible goal areas, where the routes converge either on autumn or spring migration, are indicated in blue (potential autumn goal area) or red (spring goal area). The map is a Mercator projection. [Image based on (*27*)]

Long-term tracking of individuals will also provide a basis for comparative evaluations between fish, sea turtles, and birds and for critical displacement experiments. Such displacement experiments of individuals subsequently tracked by satellite have already begun (*28*, *29*). Unraveling the entire migration history of individuals, starting as juveniles, which has not yet been achieved for any species, will reveal how an inherent spatiotemporal control program is complemented by imprinting and learning during the first and successive long-distance journeys. Another unresolved question is what determines whether migrants drift by wind and water currents or compensate for the drift. Age-dependent drift (*30*) may have important consequences for the individual migration history.

Information that is likely to be learned during the first journeys includes positions of goal areas, large-scale gradient maps as well as local site-specific maps, stopover habitat, vegetation, and food. Does this mean that migrants have a special long-term memory capacity for such information (Table 1G)? This was indeed suggested by a recent experiment (*31*) in which memory time of a particular feeding site was shown to differ between a long-distance migrant bird species (memory lasted at least 12 months) and a closely related nonmigratory species (memory retained for only some weeks). This fits well with an increase in hippocampus size from the first to the second year in the migratory species but not in the resident species (*31*). However, in spite of the special challenges of navigation and memorization, migratory passerines have smaller relative overall brain sizes than residents, a difference that may reflect the foraging challenges faced by residents during the nonbreeding season (*32*).

Analyses of the variability in individual migration history within and between populations with different migratory habits will be important to understand the evolution of new migration patterns (Table 1H). There is evidence that new migration patterns based on endogenous programs can evolve extremely rapidly, over only a few decades (*33*), but there are also opposing suggestions of evolutionary inertia, where migration routes seem to reflect the historic colonization process rather than being optimal under present conditions (*34*). Furthermore, range expansion of migratory species may be limited in comparison with residents because of constraints in the evolutionary change of genetic migratory programs (*35*).

Research about long-distance animal navigation does not, of course, only rely on tracking studies and field and behavioral experiments. Exploration of the sensory, neurobiological, and genetic mechanisms is also crucial. Current progress in revealing the mechanisms of magnetoreception is encouraging (*36*–*38*). However, we are still far from understanding how animals integrate different cues for long-distance navigation under natural conditions. Finding the spatial principles behind the details and complexities of the animals' journeys demands an unexpectedly long-lasting research effort.

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PERSPECTIVE

How and Why Do Insects Migrate?

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Countless numbers of insects migrate within and between continents every year, and yet we know very little about the ultimate reasons and proximate mechanisms that would explain these mass movements. Here we suggest that perhaps the most important reason for insects to migrate is to hedge their reproductive bets. By spreading their breeding efforts in space and time, insects distribute their offspring over a range of environmental conditions. We show how the study of individual long-distance movements of insects may contribute to a better understanding of migration. In the future, advances in tracking methods may enable the global surveillance of large insects such as desert locusts.

Large-scale movements of insects have enormous implications for human welfare (*1*), including catastrophic losses of crops (*2*), the spread of diseases to people and livestock (*3*), and the provisioning of essential ecosystem services such as crop pollination (*4*). In sheer numbers of individuals, insect migration far outweighs other migratory phenomena (*5*). Moreover, in terms of total moving biomass, the migrations of individual insect species rival and sometimes outstrip the largest extant herds and flocks of some well-known migratory mammals and birds (Table 1). And yet there is at least one fundamental difference between insect and most vertebrate migrations (*6*, *7*): As a rule, individual insects do not make a round-trip journey that returns them to the area from which they departed. Even in the case of the monarch butterfly (*Danaus plexippus*) (Fig. 1A), one of the best-studied migratory insects, few if any of the individuals wintering in Mexico return to their natal areas. Instead, monarchs repopulate northern latitudes through a process of intergenerational migration, whereby successive broods advance northward (*8*, *9*). Thus, return migration, the most common type of migration in birds and mammals (*7*), has yet to be documented in insects.

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Migration Strategies in Insects

Researchers accustomed to viewing long-range animal movements through the prism of classic return (i.e., round-trip) migration would likely categorize many so-called insect migrations as dispersal events (*6*). Entomologists, however, would disagree. Indeed, major reviews of this subject in the entomological literature have recommended abandonment of the term "dispersal" to describe insect movements; instead, all long-range movements of insects would be considered "migrations" (*1*, *7*, *10*, *11*).

Migration by vertebrates is often viewed as a mechanism for exploiting high-quality resources that are available during only a portion of the year [typically the breeding season (*7*, *10*)]. In the case of insect migrations, which we define here as repeated phenomena of directional movement that are cyclical in nature, the ultimate reasons are less clear. If insects are not able to return to a high-quality patch in a subsequent year, then why migrate at all? Although considerable progress has been made toward understanding patterns of insect migration (*1*, *11*), especially with respect to certain moths (*12*), the ultimate selection pressures resulting in these spectacular and ubiquitous movements remain mysterious. Intuitively, one expects migratory movements to evolve only when the fitness benefits exceed those of remaining in the current habitat (*13*). Whether this reasoning applies in the case of insect migrations is unclear. Because insects do not have to provide long-term care for their offspring, they can in theory reproduce in their natal area, along a migratory route, or in a discrete "winter range." This differentiates them from the classic vertebrate return-migration model. By spreading their breeding efforts both spatially and temporally, insects have the ability to "hedge their bets" by distributing their offspring across a range of areas and conditions that may be amenable for future reproduction (*14*).

To determine whether this bet-hedging hypothesis is a valid explanation for most (or any) insect migrations, one would need to know the reproductive output (and, ideally, success) of individual insects along their migratory route. Do

Table 1. Biomass of migrating animals.

Class	Species	Location	Size of herd, swarm, or flock	Biomass (tons)	Source (reference)
Insects	Darner (dragonfly), *Aeshna bonariensis*	Argentina	4–6 billion	4000	(*15*)
	Monarch butterfly, *Danaus plexippus*	Winter grounds in Mexico	100–200 million	40–80	(*39*, *40*)
	Desert locust, *Schistocerca gregaria*	Africa, Middle East, Asia	10^9–10^{11}	200,000	(*5*)
Mammals	Wildebeest, *Connochaetes taurinus*	Serengeti, Kenya and Tanzania, Africa	1.3 million	280,000	(*41*)
	Mexican free-tailed bat, *Tadarida brasiliensis mexicana*	Carlsbad Cavern, New Mexico, North America	20 million	300	(*42*)
Birds	Lesser sandhill crane, *Grus c. canadensis*	Platte River, Nebraska, North America	450,000	1440	(*43*)

individual insects simply continue to migrate until they die from exhaustion, or do they terminate their migration when reaching a suitable habitat? That is, do they simply move along a habitat cline and spread out their propagules, or do they search for a spatial peak in the reproductive landscape, as most long-distance migratory birds do (*6*)? For example, green darner dragonflies (*Anax junius*) engage in spectacular autumn migrations along the eastern seaboard of the United States (*15*). Many of the females captured in the fall are gravid, yet we do not know whether a given female is traveling to a particular site or region to lay its eggs, or whether it is slowly moving south and laying a portion of its eggs in suitable ponds along the way. There is no shortage of questions to challenge (or frustrate) researchers interested in insect migration.

Despite the importance of knowing the behavior of individual insects, the study of insect migratory movement in the field is usually only possible at the population level using radar observations (*16*), huge aerial samplers, and/or ground surveys (*17*). What seems to be emerging from several major research programs on agriculturally important pests [e.g., desert locust, *Schistocerca gregaria* (*18*) (Fig. 1B)] is that insects are often facultative migrants that respond to changes in habitat availability, quality, and level of crowding (*17*, *19*). A large majority of insects that migrate do so pre-reproductively, suggesting that such migration is in response to some indication that reproduction will be suboptimal in the natal area (*17*). This behavior may represent a multigenerational bet-hedging strategy that allows the offspring to avoid overcrowding or deteriorating environmental conditions. Spatial heterogeneity of the habitat—in particular, the existence of good and bad patches—may also select for migratory behavior as a bet-hedging strategy (*14*). However, too little is currently known about the strategies of individual insects to generalize about the ultimate factors responsible for the movements of megatons of insects within and between continents.

Fig. 1. Examples of large insects that migrate in swarms (see Table 1). (**A**) Monarch (*Danaus plexippus*); (**B**) desert locust (*Schistocerca gregaria*). [Credit: (A) U.S. Fish and Wildlife Service; (B) J. E. Estes (1939–2001), University of California Santa Barbara Geography Department]

Migration Mechanisms in Insects

At small scales, insect navigation is one of the best understood subjects in animal navigation (*20*). Far less is known about the orientation of migratory insects, however. In particular it is unclear how and whether insects decide to terminate migration, and what decision rules they employ during migration. In the case of migrating birds, a species-specific wintering ground is usually the goal, and it is located by an endogenous program of vector navigation in the first migratory journey, and possibly by "true navigation" based on experience in subsequent years (*21*). If migratory insects do not make round-trip journeys, then it is unlikely they develop navigational mechanisms based on experience (*22*). Yet, in the case of monarch butterflies, winter sites are highly localized year after year (*22*), suggesting some tight control of spatial movements across the 3000 km of North American landmass. To find their wintering sites, an environmental cue or a genetic vector engrained in an endogenous program similar to that used by juvenile migrating birds (*6*) is presumably employed.

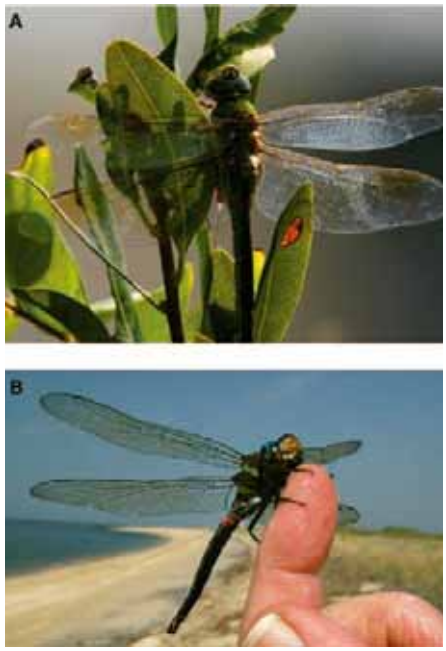

Fig. 2. Attachment of microtransmitters to migrating dragonflies (*34*). (**A**) Green darner resting on vegetation, with microtransmitter attached to ventral thorax. (**B**) Swamp darner warming up on finger before continuing migration along the Delaware Bay, New Jersey. [Credit: C. Ziegler]

Insects that migrate in the flight boundary layer (*23*), below the prevailing winds, must have a mechanism to maintain a heading. This has been proposed for monarch butterflies, with a 1° per day change accounting for the orientation of these animals through the course of a year (*9*). However, more recent data do not fully support this hypothesis (*24*). Geographical features may play an additional role in determining direction for monarchs (*24*). Orientation mechanisms to allow a migrating insect to maintain a heading have been investigated in a number of butterflies and moths and include a sun compass (*25*, *26*) and possibly a magnetic compass (*27*). Migrating butterflies and moths may also compensate for wind drift (*28*, *29*), and anecdotal evidence suggests that individuals are able even to compensate for large displacements (*30*), although this finding is controversial (*22*). The genetic control of insect migratory flight directions has yet to be confirmed.

Why We Don't Know More: The Future of Insect Migration Studies

The problems associated with studying insect migration are the same ones bedeviling anyone who wishes to study large-scale movements in a small flying animal, whether it is a bird, bat, or insect. Tracking the path of the animal over long distances has been the primary obstacle (*31*). Only recently have radio transmitters become small enough to attach to small birds and large insects (*32*) (Fig. 2), and the tracking of such animals has provided exciting new insights into migratory behavior (*31*, *33*). For example, radio tracking aided by small search planes has revealed that green darner dragonflies use falling nocturnal temperatures as a cue to initiate their southward migration (*34*). Individuals generally fly with the prevailing winds, but change or even reverse their migratory direction when encountering large geographical barriers such as ocean bays. Similar behavior in similar sites has been recorded in migratory songbirds, raising the possibility that both birds and dragonflies follow similar navigational rules (*6*).

We are entering an exciting new era for the study of insect migration. The genetic basis of insect migration can now be investigated using modern genomic methods (*35*). Ideally, lab-based experiments could be linked to field measurements of genetic polymorphisms of migratory potential that occur in certain insects; these polymorphisms, in turn, could be related to habitat heterogeneity, a

result that could allow us to link ultimate and proximate aspects of insect migration (*14*, *36*). Such studies may also provide new insights into the genetic control of migratory flight decisions per se (*9*). Biomechanical models can be used to improve our understanding of the energetic costs of insect migration (*25*, *27*–*29*), and the trade-offs those costs impose on other aspects of an insect's life history (*37*). Most important, our ability to track small animals over large distances is improving steadily. Current systems for tracking animals globally are unsuitable for creatures smaller than ~500 g, which excludes the majority of birds and bats as well as all insects. However, the signals from transmitters now being used to track dragonflies could be received from space with the installation of a small-animal tracking satellite. Such a system is technologically feasible (*38*). The ability to follow individual insects throughout their migrations will be invaluable to understanding the selective forces behind insect migration. We also need data on individual insects to understand how migratory abundance changes with climatic cycles and with the use of pesticides on targeted and nontargeted species. Until the migratory behavior of individuals can be separated out from the behavior of populations, insect migration is likely to remain a poorly understood but immensely important phenomenon.

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REVIEW

Going East: New Genetic and Archaeological Perspectives on the Modern Human Colonization of Eurasia

Paul Mellars

The pattern of dispersal of biologically and behaviorally modern human populations from their African origins to the rest of the occupied world between ~60,000 and 40,000 years ago is at present a topic of lively debate, centering principally on the issue of single versus multiple dispersals. Here I argue that the archaeological and genetic evidence points to a single successful dispersal event, which took genetically and culturally modern populations fairly rapidly across southern and southeastern Asia into Australasia, and with only a secondary and later dispersal into Europe.

Research over the past 20 years has provided an increasingly clear picture of the way in which our own species (*Homo sapiens*) emerged and subsequently spread across the rest of the occupied world. DNA evidence and fossil skeletal remains indicate that human populations that were essentially "modern" both anatomically and in their mitochondrial and Y-chromosome lineages had emerged in Africa by at least 150,000 years ago, perhaps closer to 200,000 years ago (*1*–*11*). Studies of present-day world populations (especially those based on the maternally inherited mitochondrial DNA) strongly suggest that a small subset of these African populations made the crossing from northeastern Africa, probably over the mouth of the Red Sea, and subsequently dispersed into Arabia and southern Asia sometime before 50,000 years before present (yr B.P.) (*2*, *8*, *12*–*17*) (Fig. 1). Recent studies have suggested that these populations expanded rapidly along the coastlines of southern Asia, southeastern Asia, and Indonesia to arrive in both Malaysia and the Andaman Islands by at least 55,000 yr B.P., and conceivably as early as 60,000 to 65,000 yr B.P. (*12*, *18*–*21*)—though more recent estimates of mitochondrial DNA mutation rates (*8*) suggest that these figures may be overestimates. As Carl Sauer pointed out in 1962 (*22*), a strongly coastal pattern of dispersal would make good sense in ecological and demographic terms, because this would presumably have required only limited economic adaptations from one coastal location to another.

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The subsequent dispersal southward of these populations into New Guinea and Australia (at that time connected as an extended landmass known as "Sahul") is currently more controversial. We know that typically anatomically modern populations were present at the Niah cave in Sarawak by at least 41,000 years ago (*23*), and that some of these populations had made a sea crossing of at least 90 km to reach parts of Australia by at least 45,000 yr B.P.—best represented by the typically anatomically modern skeleton from the site of Lake Mungo 3 in New South Wales (*1*, *24*–*26*). Some archaeological claims have been made for the initial colonization of Australia as early 50,000 to 60,000 yr B.P. (*27*), but these remain speculative and contested (*24*). Similarly, claims for the presence of behaviorally modern populations in Malaysia before the Mount Toba (Sumatra) volcanic "supereruption" around 74,000 yr B.P. (*16*) have yet to receive any clear support from recent archaeological research in the region (*28*). But in any event, it is clear that the initial dispersal of modern human populations eastward from their original African homeland along the so-called coastal express (*12*, *18*, *20*) route into Australasia occurred over a comparatively short time, amounting to at most 15,000 years, and probably less than 10,000 years (i.e., an overall dispersal rate of at least 1.0 km per year) if we take the combined DNA and archaeological evidence into account (*12*, *18*, *21*).

Single or Multiple Dispersals?

The most controversial issue at present centers on whether there could have been not one, but possibly two or more separate dispersals of anatomically and genetically modern humans from Africa into Eurasia. An earlier model advanced by Lahr and Foley in 1994 (*29*–*31*) envisaged at least two separate episodes of dispersal from northeastern Africa, associated with sharply differing patterns of stone-tool technology. Of these, the "northern" dispersal extended northward via the Nile Valley and the Sinai Peninsula into southwestern Asia (and eventually Europe), associated with typically blade-dominated, "Upper Palaeolithic" or "Mode 4" technology, best represented at the sites of Boker Tachtit in southern Israel and Ksar Akil in Lebanon, both dated to around 45,000 to 50,000 yr B.P. (*32*–*34*). The separate "southern" dispersal extended from the Horn of Africa across the mouth of the Red Sea (the Bab el Mandeb straits) carrying technologically simpler "Middle Palaeolithic" or "Mode 3" technology, which subsequently dispersed eastward along the coasts of southern and southeastern Asia into Australia (*21*, *29*–*31*). The sharp contrasts in the technology associated with these two dispersals were taken as an explicit reflection of two separate source populations in Africa, with the southern, Mode 3 dispersal occurring substantially earlier than the northern, Mode 4 dispersal event (*29*–*31*).

Recently, the notion of two or more separate dispersals of anatomically and genetically modern humans has come under increasing scrutiny from molecular geneticists, based on studies of both mitochondrial and Y-chromosome patterns in African and Asian populations (*2*, *8*, *12*–*15*, *18*). These recent studies suggest that the whole of modern Asian and European populations derive from one small subset of the so-called L3 mitochondrial lineage in Africa, which subsequently diverged into the derivative M and N lineages, probably shortly after their dispersal from Africa (*2*, *8*, *12*–*15*). The crux of the arguments advanced by Kivisild (*8*), Forster (*2*), Matsumura (*12*), Macaulay *et al*. (*18*), and others is that the very limited genetic diversity exhibited by modern European and Asian populations—compared to those in Africa—would be effectively impossible to reconcile with the model of two separate dispersal events, deriving from separate source populations in Africa, and hypothetically at two different dates. Essentially the same conclusion has been drawn independently by Endicott and others (*13*) based on studies of the paternally inherited Y-chromosome lineages. Even allowing for the current controversies surrounding the interpretation and dating of the DNA evidence (*2*, *5*, *8*, *11*, *14*, *35*–*37*), it is becoming increasingly difficult to reconcile the available genetic data with the hypothesis of two or more separate dispersal events from Africa into Eurasia—although this point has been debated in some earlier genetic studies (*15*, *38*).

Fig. 1. Map of possible dispersal routes of anatomically and genetically modern human populations from Africa to Asia and Australia according to Forster and Matsumura (*12*). The models assume an origin in eastern Africa, and dispersal either via the Nile Valley and Sinai Peninsula (the "northern" route) or via the mouth of the Red Sea to Arabia and Australia (the "southern" route).

The Archaeological Evidence

The major challenge of this scenario now is to document the individual steps in this colonization process on the basis of the "hard" archaeological evidence. Large areas of both Arabia and India in particular are at present largely blank areas on the archaeological map over the critical time range from ~50,000 to 60,000 yr B.P. in question (*39*, *40*). And of course, all the coastlines of this period are now deeply submerged below the rapidly rising sea levels of the past 15,000 years (*20*, *21*). There are, however, already some intriguing hints from south Asia of what future research may reveal. From the sites of Patne in western India (*41*), Jwalapuram in southeast India (*42*), and Batadomba-lena in Sri Lanka (*43*, *44*), there are archaeological assemblages showing some striking resemblances to those from eastern and southern Africa that must be from very close to the period when modern humans first dispersed from Africa (Fig. 2A). These sites contain large numbers of small "crescentic" forms of stone tools (evidently parts of hafted implements, and conceivably components of archery equipment) (*45*, *46*) that are markedly similar to those that define the so-called Howiesons Poort technology in southern and eastern Africa, dated broadly to around 55,000 to 65,000 yr B.P. (*45*–*51*)—as at the sites of Mumba in Tanzania (*45*, *52*), Norikiushin in Kenya (*53*), and a range of similar sites in southern Africa (*45*–*49*) (Fig. 2B). Broadly similar industries including rather larger forms of backed "segment" forms are dated to between 60,000 and 40,000 yr B.P. at the site of Enkapune ya Muto in Kenya (*53*, *54*). Although

the Indian crescentic tools are generally smaller than those from the African sites, the sequences at Patne and Jwalapuram suggest that these forms become increasingly larger in the lower levels of the sequence, and correspondingly more similar to the African tools (*41*, *42*). Even more strikingly, these Indian sites have produced carefully shaped and perforated beads manufactured from fragments of ostrich eggshell, closely similar to those found in African sites (*39*, *41*, *45*, *47*), together with a further piece of ostrich eggshell incised with a distinctive criss-cross motif (*41*), which is strongly reminiscent of designs engraved on pieces of red ochre from the later Middle Stone Age levels in the Blombos Cave in South Africa (*55*) (Fig. 2, A and B), together with similar designs incised on fragments of ostrich-eggshell water containers from the site of Diepkloof in the western Cape (*56*), dated respectively to ~75,000 and 60,000 yr B.P. (*50*, *51*). At present, the Indian and Sri Lankan sites in question can only be reliably dated back to around 34,000 yr B.P. (in calibrated radiocarbon terms) (*34*, *39*, *41–44*), but current excavations at the Jwalapuram site in southern India suggest that similar technologies may go back to a much earlier date (*42*). Unless these striking similarities in material culture are entirely coincidental, they point strongly to a direct connection between the earliest modern human colonists in southern Asia and their probable ancestors in eastern and southern Africa.

The Australian Archaeological Record

The greatest enigma in the current archaeological record lies in the lack of similarly "advanced" technologies in the areas to the east of the Indian subcontinent, and especially in the relatively well-explored areas of Australia and New Guinea, which were colonized by anatomically modern humans from at least 45,000 yr B.P. onward, as discussed above (*24–26*). The earliest stone-tool technologies documented across the whole of Australasia are conspicuously lacking in any trace of distinctively "modern" or "Upper Palaeolithic," blade-based technologies of the kind recorded from both the later African Middle Stone Age sites and the earliest modern human sites in southwest Asia and Europe (*26*, *57*, *58*). These Australian technologies consist of very simple, flake-based industries, completely lacking in typical blade forms and apparently with little or no trace of typically Upper Palaeolithic tool forms such as end scrapers, backed blades, or burins—a pattern of technology that persisted in Australia from at least 45,000 yr B.P. down to the middle of the Holocene period, around 5000 to 7000 yr B.P. (*26*, *57*, *58*). How can we reconcile this observation with the hypothesis that these technologies developed from more "advanced," blade-based technologies in Africa (and apparently parts of India) with the initial dispersal of anatomically and genetically modern populations in their eastward migration?

The answer to this paradox might lie partly in environmental factors, and partly in the patterns of cultural and technological development that are probably inherent in the progressive dispersal of small-scale human populations across a long and environmentally complex colonization route. Three factors in particular are likely to have been significant in this context. The first, and potentially most important, factor lies in the general scarcity of high-quality, fine-grained stone for tool production in most areas of eastern and southeastern Asia (*59*, *60*). Blade technology in particular is heavily dependent on the availability of nodules of fine-grained stone such as flint, chert, obsidian, or other fine-grained rocks, which are scarce over many

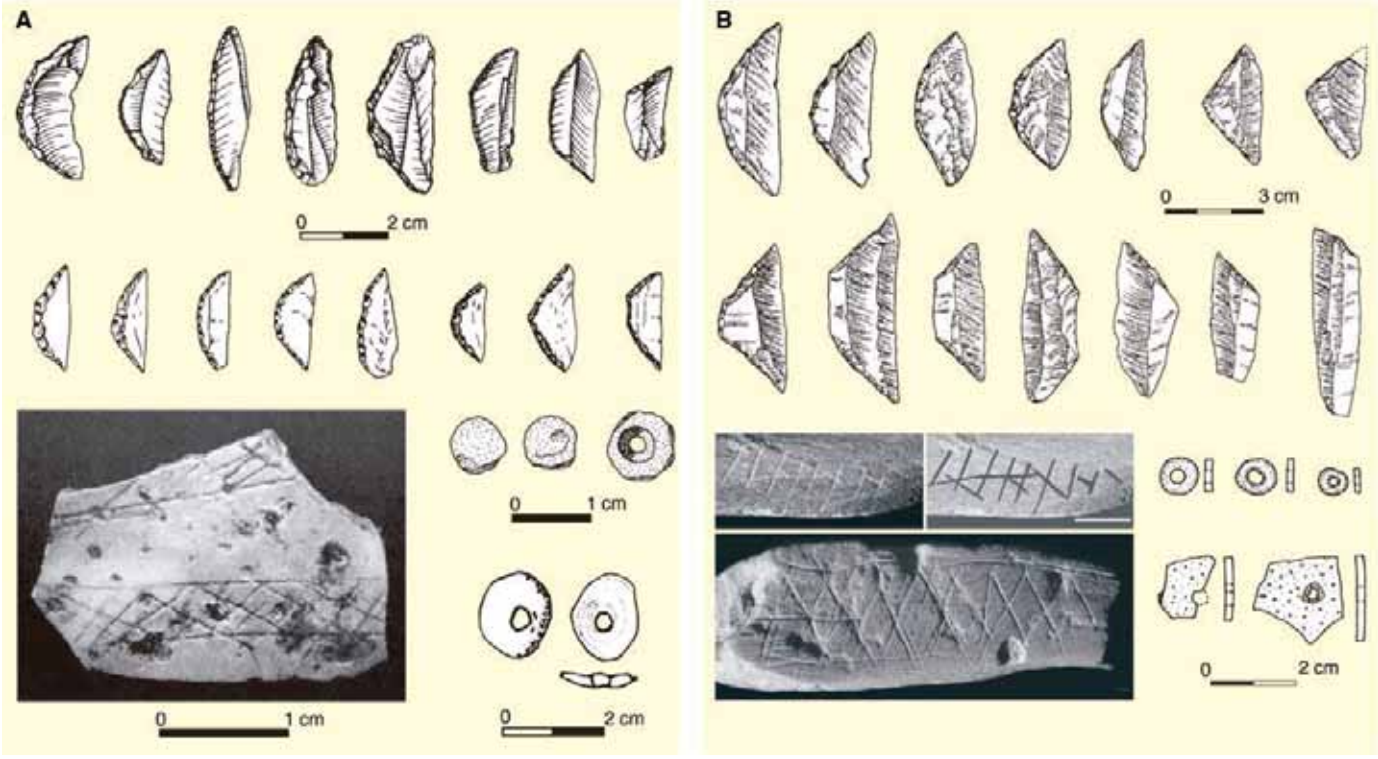

Fig. 2. (**A**) (Upper) Assemblages of "crescentic" and related stone tool forms from the sites of Patne in western India (*41*) and Batadomba-lena in Sri Lanka (*43*), radiocarbon dated to between 30,000 and 34,000 (calibrated) yr B.P. (Lower) Geometric design engraved on ostrich eggshell fragment from Patne (left) and specimens of ostrich eggshell beads (and preforms) and a perforated shell recovered from Patne and Batadomba-lena, respectively. (**B**) (Upper) Crescentic and related tool forms from the "Howieson's Poort" levels at Klasies River (South Africa) dated to ~60,000 to 65,000 yr B.P. (*48*). (Lower) Geometric designs incised on pieces of red ochre from the Blombos Cave, South Africa (~75,000 yr B.P.) (*55*) and ostrich eggshell beads from the site of Enkapune ya Muto, Kenya (~40,000 yr B.P.) (*56*).

areas of eastern Asia. Even if there were occasionally localized areas of better-quality stone, such as obsidian or high-quality cherts, the patterns of lithic technology would inevitably have to "adapt" to the more extensive areas in which these better-quality raw materials were lacking. Indeed, it has often been suggested that the technology over large areas of eastern Asia may have been much more dependent on wood, or even bamboo, for tool manufacture than on conventional stone tool supplies (*59*, *60*).

The second major factor in stone tool technology lies in the specific functions for which the tools were required. If, as most of the current models suggest, the initial colonization of southeastern Asia and Australasia followed a primarily coastal route (*12*, *18*, *20*, *21*, *61*), then the technologies would be likely to adapt primarily to the exploitation of coastal resources, such as fish, shellfish, and marine mammals (together with tropical plant foods) with perhaps only a minor component of hunting larger land mammals, of the kind that clearly formed a major part of the human economy in both Africa and the whole of western Asia and Europe (*21*, *59*). This would presumably have involved much less emphasis on various forms of hunting equipment (such as spears, meat-processing tools, etc.), as well as equipment involved in the manufacture of elaborate skin clothing, or the construction of tents and other living structures, that were essential to survival in much colder, more northerly environments (*59*, *62*).

Finally, there is the more fundamental demographic and evolutionary issue of the repeated founder effects, and associated cultural drift, as relatively small population units expanded progressively eastward along the southern and southeastern Asian coasts (*21*, *63*). These repeated, successive, and cumulative small-scale founder effects would inevitably operate not only on the biological and genetic features of the populations (*6*, *7*, *11*) but also on their repertoires of cultural and technological behavior—probably leading to a progressive loss in the complexity and diversity of cultural and technological patterns with increasing distance from their demographic point of origin. Arguably the most striking illustration of this kind of founder effect and technological drift process can be seen in the loss of several technological features (such as fishing, bone tools, and other cultural elements) associated with the settlement and cultural development of Tasmanian populations, following their initial colonization of the island around 35,000 yr B.P. (*64*–*66*). All of these cultural and technological processes could be seen as a direct parallel to the progressive loss in the genetic diversity of the dispersing modern human populations over geographical trajectories extending from their putative African origin progressively eastward and westward—as recently documented in studies of present-day genetic patterns by Prugnolle *et al.* (*6*), Liu *et al.* (*7*), and others (Fig. 3).

When viewed in these terms, the relative "simplicity" of the technology associated with the initial modern human settlement of southeast Asia and Australia becomes not merely plausible but arguably largely predictable, in demographic and cultural terms. Although these early Australian technologies are strikingly different from those of the early Upper Palaeolithic, blade-dominated industries in southwestern Asia and Europe, there is nothing distinctively Middle Palaeolithic (or Mode 3) about their character (*29*–*31*). The whole of the Australian technology is conspicuously lacking in anything resembling typically Middle Palaeolithic "Levallois" or similar "prepared core" techniques (*26*, *57*, *58*), and the kinds of simple retouched flake tools encountered in these industries could be paralleled just as easily in some of the early Upper Palaeolithic industries in Europe (*67*) as in the Eurasian and African Middle Palaeolithic/Middle Stone Age sites. More specifically, it could be argued that the curious, single-platform "horse-hoof" cores that are such a distinctive feature of the earliest Australian industries (such as those from Lake Mungo in New South Wales) (*26*, *57*, *58*, *68*) have more in common with simplified forms of single-platform blade cores (Fig. 4) in their basic conceptual and flaking strategies than with the distinctively "radial" or "centripetal" patterns of flaking, which are the hallmark of the African and Eurasian Middle Palaeolithic prepared core techniques (*59*, *69*). Any suggestion that the earliest colonists of southeast Asia and Australia carried with them distinctively Middle Palaeolithic, Mode 3 technologies, as suggested in the "multiple dispersals" model (*29*–*31*, *39*), would seem to have little support in the documented archaeological record from Australia. The totally "modern" character of the burial rituals, personal ornaments, abundant use of red ochre, and elaborate ground and shaped stone axes, documented from effectively the earliest stages of colonization of Australia (*26*, *57*, *58*, *70*), should also be kept in mind in this context. As several authors have pointed out in relation to the 100,000-year-old ritualistic burials from the sites of Skhul and Qafzeh in Israel (*17*, *71*–*73*), there is clearly much more to the emergence of cognitively "modern," symbolically constructed behavior than the production of typically Upper Palaeolithic stone tools.

The Colonization of Western Asia and Europe

One important implication of this single-dispersal, "southern route" colonization of Eurasia from

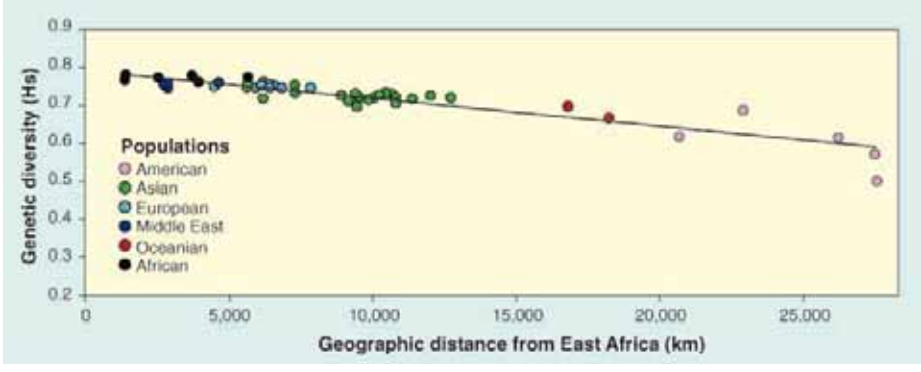

Fig. 3. Decline in the genetic diversity of present-day human populations with increasing distance from the presumed point of dispersal of anatomically and genetically modern populations in East Africa. [Reprinted from Prugnolle *et al.* (*6*), with permission from Elsevier.]

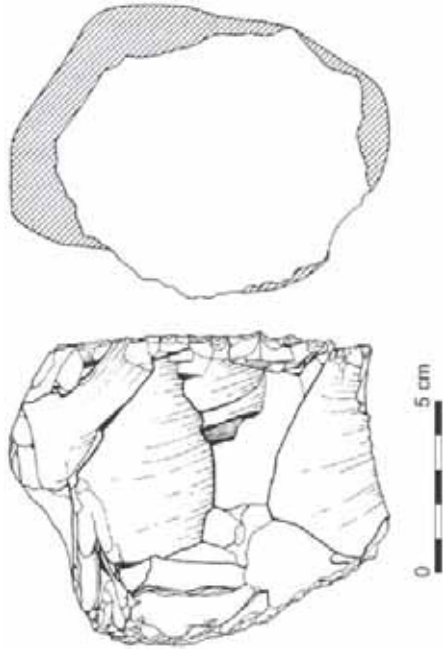

Fig. 4. "Horse hoof" core from Lake Mungo, Australia, dated to ~40,000 to 45,000 B.P. The basic flaking strategy of these cores is analogous to that of simplified forms of single-platform blade cores (*26*).

eastern Africa is that the modern human colonization of the Near East and Europe must have reached these areas via western or southern Asia, rather than via the Nile Valley and Sinai Peninsula, as implied in the "northern" dispersal route. This conclusion receives further support from the relatively late dating of the arrival of modern humans in Europe (~40,000 to 45,000 yr B.P.) implied by both the archaeological and DNA evidence (*9*, *34*, *74*, *75*), as compared to the earlier colonization of southern and southeastern Asia implied by both the DNA evidence and the early archaeological and skeletal evidence from Australia. Some archaeological support for the northern route could perhaps be seen in the presence of early blade industries at sites such as Taramsa in the Nile Valley (*76*), but these sites are only tentatively dated at present, and the industries show at best only tenuous morphological similarities to those from the 45,000- to 50,000-year-old sites of Boker Tachtit and Ksar Akil in the Near East (*32*, *34*, *76*).

The alternative possibility would be to see the origins of the Near Eastern industries as related directly to the single, southern dispersal event, which carried modern humans first across the Bab el Mandeb straits to Arabia, followed by a splitting of the migration routes, one moving eastward toward India, and the other moving northward, either through central Arabia or the eastern coast of the Red Sea into the Near East (*8*, *13*, *14*, *16*, *21*)—with an associated divergence of technological patterns during the course of these dispersals. Alternatively, this split could have occurred rather further to the east in, say, eastern Arabia or Iran (*14*, *16*, *21*). The character of the industry from the basal ("Endingi") levels in the Enkapune ya Muto rock shelter in Kenya, dated to ~50,000 to 60,000 yr B.P. (comprising elongated Levallois points and blade forms, reminiscent of those in the earliest Near Eastern industries) (*54*), could perhaps provide some support for this model.

But until the character of the archaeological evidence from both the Nile Valley and Arabia is better documented (*40*, *76*), the precise source of the earliest modern human colonists in the Near Eastern zone will be difficult to pin down. And the evidence for the earlier, unsuccessful dispersal of anatomically modern humans from North Africa into the Near East around 100,000 years ago (as represented by the finds from Skhul and Qafzeh in Israel) must make us aware that not all modern human dispersal events were necessarily successful in either the long or short term, or in terms of their continuity in the subsequent human DNA record (*1*, *2*, *17*, *29*, *32*). Even if the broad outlines of the modern human dispersal from Africa into Eurasia are now becoming much clearer, in terms of both the genetic and archaeological evidence, the finer details of this colonization process have still to be worked out.

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Single-Molecule, Motion-Based DNA Sequencing Using RNA Polymerase

William J. Greenleaf[1] and Steven M. Block[1,2]*

Traditional, dideoxy-based (Sanger) sequencing of DNA is remarkably reliable and robust. However, the quest for more rapid, economical ways to sequence genomes has driven interest in alternative approaches (*1*, *2*). Methods capable of sequencing single DNA molecules represent the logical endpoint of miniaturization, leading to the maximum extraction of information from a minimum of material.

Most approaches to single-molecule sequencing have concentrated either on the detection of fluorescent nucleotides incorporated during DNA polymerization or on the conductance changes produced as DNA molecules traverse membranes through nanopores. For the present, both these approaches are beset by limitations. By using fluorescence signals collected from individual molecules, one group reported sequencing up to five noncontiguous bases on specially designed templates (*2*), and nanopore techniques have distinguished base differences only in the context of engineered hairpins (*3*) or identified DNA polymers composed of long stretches of a single nucleotide species (*4*).

Here, we report a method for sequencing that relies upon resolving the motion of a processive nucleic acid enzyme. We used a newly developed assay for tracking transcription by single molecules of *Escherichia coli* RNA polymerase (RNAP) in which a pair of optical traps levitates two polystyrene beads: one attached to the RNAP enzyme and the other to the distal end of a DNA template. Transcriptional motion of RNAP along the template changes the length of the DNA tether joining the two beads, leading to displacements that can be registered with angstrom-level precision, affording single–base pair resolution (*5*). When the transcriptional assay is carried out in a buffer where one of the four nucleoside triphosphates (NTPs) is present at a very low concentration, RNAP will be induced to pause at every DNA position that requires the addition of the limiting nucleotide.

To sequence DNA, we repeated the single-molecule assay four times (on four copies of the target DNA sequence) with each NTP species held rate-limiting in turn, and we inferred the template sequence directly from the ordered sequence of pauses in the set of four transcription records. The success of this enterprise relies on being able to align all four records to within one base pair. To establish subnanometer alignment, we used known sequence information found in the DNA regions flanking the unknown segment to be sequenced, which produces an expected pause pattern. This pattern was used to place the four records in register by a maximum correlation method (*6*, *7*). The flanking sequences used for alignment provide a common starting point and play an analogous role to the oligonucleotide primers used in Sanger sequencing.

Four aligned records are shown (Fig. 1A). DNA positions from these records were histogrammed, smoothed, and normalized (Fig. 1B). Bases were assigned to every 3.4 Å window [corresponding to the distance spanned by 1 base pair (bp)] by following a simple heuristic. First, if a single histogram peak was detected within one of the windows, it was assigned to the corresponding base. Next, for windows with multiple peaks, the tallest peaks were associated with the nearest unassigned windows. Last, any remaining windows were assigned to the base with the highest histogram value found at the center of the window. With this scheme, we correctly identified 30 out of 32 bases in a target region on the basis of less than 3 min of net observation time for exactly four molecules (Fig. 1). Greatly improved accuracy can be obtained by combining statistics from multiple single-molecule records and by using more a sophisticated base-calling algorithm, e.g., one based on peak deconvolution.

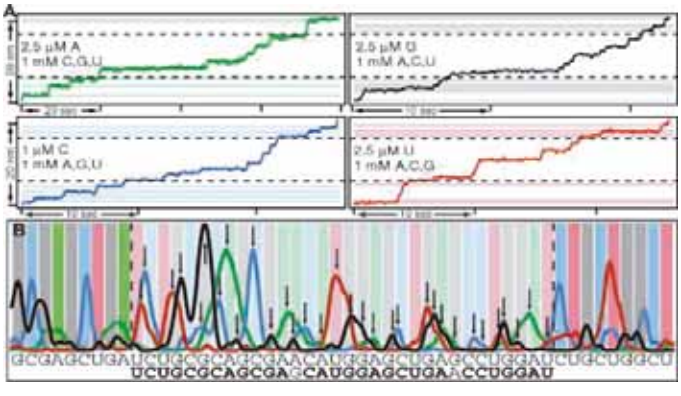

Fig. 1. Motion-based DNA sequencing. (**A**) Aligned records of transcriptional position versus time for a single molecule of RNAP under the four different limiting nucleotide conditions (ATP, green; CTP, blue; GTP, black; and UTP, red). Positions of expected pauses used for record alignment (solid horizontal lines) flank the region to be sequenced (dotted horizontal lines). (**B**) Position histograms for the data in (A), normalized and smoothed. Flanking positions used for alignment (dark vertical bars) and unknown bases to be called (light vertical bars) are shown; base calls are indicated (arrows). The true sequence of the template is shown above the inferred sequence, with 30 of 32 correct bases (boldface type).

Read lengths of DNA sequences determined by this approach are ultimately limited, in principle, by the processivity of RNAP, which is thousands of base pairs. In practice, it has proved possible to follow RNAP at the single-molecule level with near–base pair accuracy over templates in excess of 2000 bp (*6*). Another factor potentially influencing read length may be transcriptional pausing. About 95% of pauses are brief (~1 to 5 s) and sequence-specific and occur about once every 100 bases (*6*): These are not likely to pose problems. However, ~5% of pauses exceed 20 s and occur at random positions, about once per 1000 bases: These are associated with misincorporation errors. Because such errors are unrelated to any particular sequence, records from multiple molecules may be combined to disambiguate these events from pauses induced by limiting NTPs. DNA reading speeds will ultimately be set by RNA synthesis rates at limiting NTPs (several bases/s). However, the degree to which motion-resolved sequencing can be parallelized and/or miniaturized to obtain signals from many molecules remains to be established. This proof of principle demonstrates that the movement of a processive nucleic acid enzyme may be used to extract sequence information directly from DNA and opens the door to further refinements and extensions of the technique.

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Supporting Online Material

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Materials and Methods
References

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Climate-Controlled Holocene Occupation in the Sahara: Motor of Africa's Evolution

Rudolph Kuper and Stefan Kröpelin*

Radiocarbon data from 150 archaeological excavations in the now hyper-arid Eastern Sahara of Egypt, Sudan, Libya, and Chad reveal close links between climatic variations and prehistoric occupation during the past 12,000 years. Synoptic multiple-indicator views for major time slices demonstrate the transition from initial settlement after the sudden onset of humid conditions at 8500 B.C.E. to the exodus resulting from gradual desiccation since 5300 B.C.E. Southward shifting of the desert margin helped trigger the emergence of pharaonic civilization along the Nile, influenced the spread of pastoralism throughout the continent, and affects sub-Saharan Africa to the present day.

During the past decade, it has become increasingly clear that climate signals extracted from polar ice caps and ocean floor sediments cannot be directly translated into climate and environmental fluctuations on tropical and subtropical continental regions habitable by humans. Contrary to the concept of the Holocene as a climatically stable period (*1*), all geological and archaeological evidence from the hypercontinental Eastern Sahara indicates marked climatic and environmental changes over the past 12,000 years that often do not reflect climate anomalies recorded in high-latitude archives (*2*). The Eastern Sahara covers >2,000,000 km^2 and includes the Western Desert of Egypt, Northwest Sudan, and the adjacent parts of Libya and Chad, which together are about the size of western Europe. Today, it is the largest hyper-arid warm desert on Earth, with virtually no rainfall (<2 mm/year) in its center and maximum precipitation of 30 mm/year at its peripheries against potential evaporation rates of up to >6000 mm/year (*3*).

As a consequence of the extreme aridity and scarceness of wells, the Eastern Sahara—outside the Nile valley and groundwater-supported oases in the Egyptian "New Valley," Fayum, and Siwa—has been completely void of permanent human settlement in recent millennia. For this reason, it is a unique natural laboratory for the reconstruction of the links between changing climate and environments, and human occupation and adaptation, with prehistoric humans as sensitive indicators of past climate and living conditions. Their mere presence there, documented in countless archaeological remains and occupation sites, or their absence, serves as unfailing evidence for shifting climatic zones, as well as the development of Neolithic achievements, interregional contacts, and innovative strategies that have modeled the sociocultural evolution on the African continent to the present day.

Understanding the spatial and temporal variations of past rainfall requires integration of geological, archaeological, archaeobotanical, and archaeozoological field data into regional chronologies at several time slices and distinct latitudinal zones. A vast region, stretching 1800 km from latitudes 15°N to 31°N and 1300 km between longitudes 22°E and 34°E, can hardly be treated as a single entity. Such a multi-indicator and supraregional approach prevents overstressing of local stratigraphies that may not necessarily reflect conditions in the same geographical latitude (*4*) and avoids the ambiguity of condensing geological and biological evidence from several millennia into a single age loosely defined as "Holocene optimum" (*5*, *6*).

Onset of humid conditions. During the Alleröd interstadial (about 11,900 to 10,800 B.C.E.), when Northwest Europe witnessed temporarily waning ice sheets, and the following Younger Dryas, the final cold phase of the Pleistocene, the Eastern Sahara was still void of any aquatic environments and as hyper-arid as it was during the Last Glacial Maximum at 20,000 B.C.E. The first signal of a changing climate occurred in the early Preboreal with the establishment of postglacial conditions in the mid-latitudes, supported by evidence of a sudden appearance of carbonate lake formations in the Sudanese Sahara and of siliceous mud deposits in the Egyptian Sahara.

Radiocarbon dates of the base levels of these paleolakes and playa-type rain pools reveal the almost contemporaneous onset of pluvial conditions between latitudes 16°N and 24°N at about 8500 B.C.E., indicating an abrupt northward shift of the tropical rainfall belts over as much as 800 km within just several generations (*7*, *8*). This decisive climate change can be attributed to tropical summer rains owing to a major extension of the paleomonsoon system, whereas the contribution of Mediterranean winter rain systems north of 24°N remains vague. As a result, notably improved environmental conditions spread over the entire Eastern Sahara (*7*–*15*), with semihumid climates in its southern part and semi-arid conditions in its center.

Time transgressive drying of the Eastern Sahara. The chronology of radiocarbon dates from early and mid-Holocene occupation sites along a north-south transect through the Eastern Sahara provides a spatial and temporal synthesis of the directional trend in shifting human populations (Fig. 1 and fig. S1). It was compiled from almost 500 radiometric results from about 150 excavations at non-oasis sites, supplemented by condensed chronologies for Nabta and Kiseiba (*4*), the Egyptian oases (*16*, *17*), and the Nile valley (*18*). The general array of radiocarbon dates, with older dates in the north and the bulk of younger dates in the south, clearly indicates (i) a movement of prehistoric populations toward the present-day Sahelian zone; (ii) a dearth of early Holocene data from the Nile valley at a time when human presence in the Eastern Sahara is well documented; and (iii) a sharp break of settlement in the Egyptian Sahara at about 5300 B.C.E. (except for some ecologically favored refuges such as the Gilf Kebir Plateau), the time when Neolithic and predynastic farming communities began flourishing in the Nile valley.

Phases of human occupation. Cumulative curves of the archaeological chronological data (Fig. 2) indicate four distinct occupation phases: (i) the Reoccupation phase (8500 to 7000 B.C.E.), starting with surprisingly early settlement in the Egyptian Sahara; (ii) the subsequent Formation phase (7000 to 5300 B.C.E.), ending abruptly in all areas without permanent water; (iii) the Regionalization phase (5300 to 3500 B.C.E.), featuring retreat to highland refuges with continuing rains and temporary lakes; and (iv) the Marginalization phase (3500 to 1500 B.C.E.), with only transient human activities in the Egyptian Sahara and prehistoric occupation restricted to Northern Sudan.

Here, we discuss these major occupation phases in the context of their assumed environmental settings. We provide the climatic background in synoptic zones, limited by best estimate isohyets (lines of equal annual precipitation) on the basis of geological, archaeozoological, and archaeobotanical data (*7*, *9*–*12*, *19*). Correlation between the proposed pluviometric pattern and the archaeological evidence produces a coherent scenario for environmental, sociocultural, and economic change in the Eastern Sahara since the terminal Pleistocene.

At that time, the Saharan desert extended about 400 km farther south than it does today, covering more than one-third of the African continent (Fig. 3A and fig. S2A). Prehistoric sites along the Nile are overrepresented at Lake Nubia (because of the archaeological rescue missions related to the

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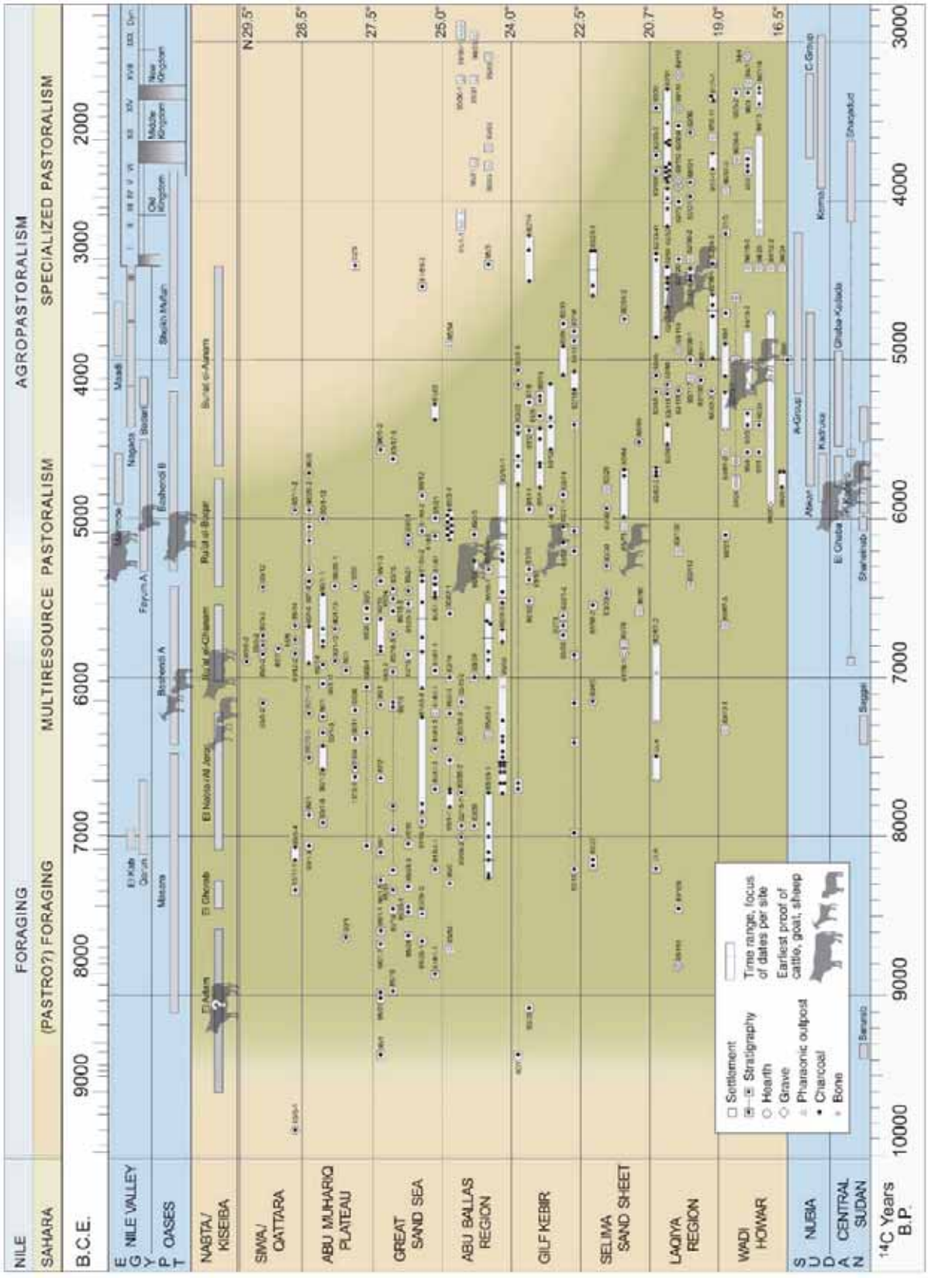

Fig. 1. Radiocarbon dates from early and mid-Holocene occupation sites in the Eastern Sahara. The graph is arranged from north to south and based on almost 500 radiometric results from our excavations of non-oasis prehistoric sites, with condensed chronologies for the Egyptian sites of Jebel Nabta and Bir Kiseiba (*4*), and the Egyptian and Sudanese Nile valley and oases (*16*–*18*). The data show the clear trend of southward shifting occupation driven by the retreat of monsoon rainfall, and the contrasting economies in the Nile valley and the Sahara. Green shading marks humid conditions; symbols of domesticated cattle demonstrate the spread of pastoralism. See fig. S2 for details and site locations. Years B.P., years before present; Dyn., dynasty.

Aswan high dam) but contrast clearly with the complete lack of evidence from the desert. During the terminal Pleistocene "Wild Nile" stage at about 12,000 B.C.E (*20*), living conditions along the river became harsh and caused conflicts for land and food, as indicated, for example, by the late Paleolithic Nubian cemetery of Jebel Sahaba, in which many of the buried individuals died a violent death (*21*).

Early Holocene reoccupation (8500 to 7000 B.C.E.). With the rapid arrival of monsoon rains at 8500 B.C.E., savannah-like environments turned the Eastern Sahara into a habitable region, and prehistoric humans soon settled there (Fig. 3B and fig. S2B). Groups from the south, already adapted to savannah ecology, extended their traditional way of life following the northward shifting rains, whereas Nile dwellers may have left the inhospitable valley. The epipaleolithic tool kit, as well as archaeozoological evidence from Nabta and Kiseiba, defines them as hunter-gatherers, possibly already practicing some animal husbandry (*4*). While this pastro-foraging economy needs further confirmation, "wavy line" decorated pottery—the first ceramics in the Old World—is a key African achievement of the ninth millennium B.C.E. (*22*).

Epipaleolithic camp sites in the Regenfeld area dated to 8000 to 7000 B.C.E. demonstrate quick migration of populations over several hundreds of kilometers into the central Great Sand Sea, where they encountered satisfactory living conditions in what is today the Libyan Desert's most barren part (*23*). Rains had turned the late Pleistocene dunes into pasture that provided wild grains for the hunter-gatherers and browsing for their game. Most notable is the almost complete lack of settlements in the Egyptian Nile valley, with the exception of El Kab (*24*). The dearth of archaeological sites along the Nile and in the Wadi Howar region reflects conditions too marshy and hazardous for settlement. During the early Holocene humid optimum, hunters and gatherers obviously preferred the less wooded grassland farther north to the regularly flooded and densely wooded environments of the southern Sahara.

Mid-Holocene formation (7000 to 5300 B.C.E.). After 7000 B.C.E., human settlement became well established throughout the Eastern Sahara by way of economical and technological adaptations to regionally different ecological requirements (*25*) (Fig. 3C and fig. S2C). On the Egyptian Abu Muhariq Plateau, bifacial technology obviously rooted in the Levant caused a complete change in the lithic tool kit that can be traced into the later predynastic cultures of the Nile valley (*26*). Impression-decorated pottery of Sudan tradition, on the other hand, is represented as far north as the Egyptian oases and the Great Sand Sea (*27*). The most important achievement of this phase is the introduction of domestic livestock. Sheep and goats, for which an early record also exists in Egypt's Eastern Desert (*28*), must have been introduced from their wild progenitors in western Asia (*29*), whereas cattle appear to have been domesticated locally. Livestock keeping, well documented at Nabta Playa (*4*), for example, became an essential component of a multi-resource pastoral economy that marks the beginning of African pastoral societies. Depending on local factors, their economic base differed substantially. In the western Abu Ballas area (Mudpans site 85/56), for example, rich faunal material from about 6400 B.C.E. did not reveal any evidence of domestic livestock (*30*), whereas in the eastern part (Eastpans site 96/2) cattle are well documented, together with a new type of undecorated late Neolithic pottery (*25*).

The radiocarbon dates do not indicate any rupture in regional climatic development between 7000 and 5300 B.C.E. The disparity in ceramics and lithic artifacts at Djara and Mudpans at 6000 B.C.E. suggests a break between two phases ("A" and "B"), which coincides with the arrival of sheep and goats (*26*). Some cultural changes may consequently have occurred beyond climate control.

According to the deficiency of occupation sites, regular monsoonal rains have ceased to reach the Egyptian Sahara not later than 5300 B.C.E. At Djara and on the Abu Muhariq Plateau there is a substantial decline in radiocarbon dates (*25*). Another abrupt end of occupation is observed in the central Great Sand Sea, whereas the few younger dates from Abu Minqar may be linked to local springs and transhumance from the oases depression (*27*). A comparable pattern of semi-nomadic occupation underlies the evidence for cattle at Eastpans 96/2, when living conditions in the more distant parts of the Abu Ballas region had already deteriorated. With the end of the Formation phase at 5300 B.C.E., multi-resource pastoralism appears to have become the vital human subsistence strategy in the Egyptian Sahara while at the same time the first farming communities developed in the Fayum.

Mid-Holocene regionalization (5300 to 3500 B.C.E.). The retreat from desiccating regions into ecological niches such as the Gilf Kebir and the beginning exodus to the Sudanese plains, where rainfall and surface water were still sufficient (Fig. 3D and fig. S2D), fostered more regionally diverse sociocultural adaptations. The few dates from the western fringes of the Great Sand Sea, the Abu Ballas area, and the Abu Muhariq Plateau reflect only sporadic occupation, whereas the eastern Abu Minqar and Abu Ballas areas lie within the range of transhumance from the Farafra and Dakhla oases. Certain ceramic

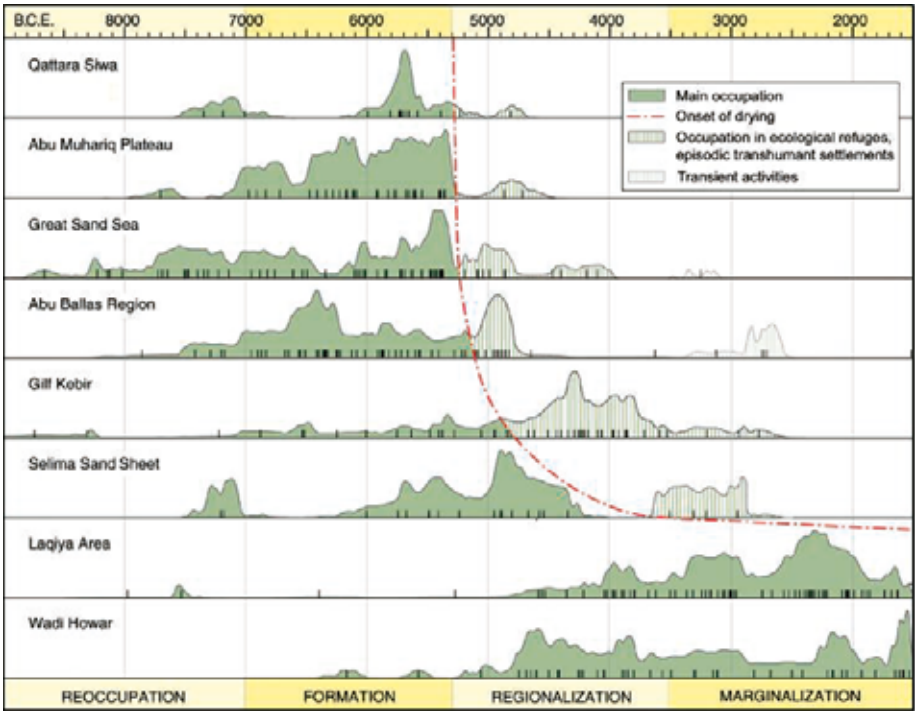

Fig. 2. Major stages of early and mid-Holocene occupation in the Eastern Sahara based on the cumulative curves of calibrated radiocarbon dates from 150 archaeological excavations. Regions are arranged from north to south. The Reoccupation phase (8500 to 7000 B.C.E.) is characterized by early settlements in the northern regions at the beginning of the Holocene humid optimum. Major occupation continues during the Formation phase (7000 to 5300 B.C.E.) until the onset of arid conditions in the Egyptian Sahara. The Regionalization phase (5300 to 3500 B.C.E.) is characterized by the retreat of populations to ecological refuges such as the Gilf Kebir plateau, seasonal or episodic transhumance, and a marked migration into the Sudanese Sahara. During the Marginalization phase (3500 to 1500 B.C.E.), Southwest Egypt receives only passing visits while prehistoric occupation in Northern Sudan persists until the end of humid conditions at 1500 B.C.E.

traditions that originated in the Gilf Kebir later occurred in the Laqiya region of Northern Sudan (*27*), where progressive southward movement is reflected, for example, by the distribution of distinct grinding implements (*30*). The previously ubiquitous "wavy line" pottery is replaced by more local pottery styles. Of particular importance is the rise of specialized cattle pastoralism (*4*, *31*), which later became a basic way of life throughout sub-Saharan Africa. This Saharan path to a productive economy was a specific African variant of a crucial change in human evolution, contrasting with the traditional Near East model of Neolithization. In place of the transition from nomadic hunter-gatherers to sedentary, pottery-producing farmers and livestock keepers, evidence suggests that largely sedentary and pottery-producing hunters, fishers, and gatherers became nomadic cattle herders. Cereal farming does not seem to have been a constituent of this Saharan "Neolithic revolution," given that the mid-Holocene savannah still provided sufficient wild-growing grains, fruits, and tubers.

Paradoxically, in certain landscapes the decreasing trend in annual precipitation may have been associated with an increase in the vegetation cover because of a change in seasonality. Geoarchaeological evidence from the Gilf Kebir suggests that the intense daytime summer monsoon rains of the early Holocene pluvial resulted in less grass growth than the quantitatively lesser winter rains of the terminal humid phase, which presumably fell at night (*32*). These favorable circumstances may have maintained the rich culture of cattle keepers depicted in the rock art of the Jebel Ouenat and Gilf Kebir.

The large-scale exodus from the Egyptian Sahara coincides with the rise of sedentary life along the Nile. The first Neolithic communities in Fayum and Merimde, starting around 5000 B.C.E. with already fully developed cultivation of wheat and barley, are clearly rooted in Near East traditions. At the same time, essential social and cognitive aspects can be traced back to Saharan cattle herders and their spiritual heritage. Neolithic settlements of the Badari culture in the Nile valley recall African livestock enclosures and suggest a rather mobile existence (*33*). The practice of cattle burials is a presumably religious custom that has been recorded in the Egyptian Sahara from the fifth millennium B.C.E. (*4*). Saharan traditions of cattle pastoralism have thus become an essential component of Neolithic life in the Nile valley.

Late Holocene marginalization (3500 to 1500 B.C.E.). After 3500 B.C.E., rains ceased even in ecological niches such as the Gilf Kebir, and permanent occupation was restricted to southern areas such as Laqiya (*34*) and Wadi Howar in Northern Sudan (fig. S2E). For the pharaonic empire, well established after 3000 B.C.E., the Western Desert obviously played a marginal role. Generally considered a "country of evil and death," it was thought to bar the Egyptian Nile valley from the Sudanese Sahara, where cattle herders still practiced their Neolithic lifestyle. Sporadic finds of Egyptian pottery near Laqiya (*27*) support rare historical reports about desert journeys during the sixth Dynasty that were considered as daring advances into the unknown.

Recent discoveries, however, shed new light on pharaonic activities in the Egyptian Sahara. Besides an elaborate desert station of King Khufu, the builder of the great pyramid, 30 outposts between Dakhla and the Gilf Kebir indicate the first trans-Saharan trail into the interior continent (*35*–*37*). At first related to Ain Azil, Ancient Egypt's westernmost town in Dakhla (*38*), and then throughout dynastic times, these desert stations indicate watch-posts concerned with prospecting or trading, or the prevention of smuggling, of African goods to the Nile valley. Because the camel was introduced to Africa only during the first millennium B.C.E., any long-distance travel through waterless desert had to rely on donkeys. Their water needs required extraordinary logistical skills and geographical knowledge—an example of how early societies coped with the challenges of hyper-arid environments.

Conclusions. Whereas earlier studies have dealt with the response of discrete cultures to climate changes in distant regions during different periods (*39*, *40*), we present here a consistent model of how past climate changes, over a coherent region of subcontinental scale, have affected human societies throughout the Holocene. Contrary to inferences from offshore marine sediment records and numeric modeling (*41*, *42*), the only supraregional climate signal in the geological and archaeological archives of the

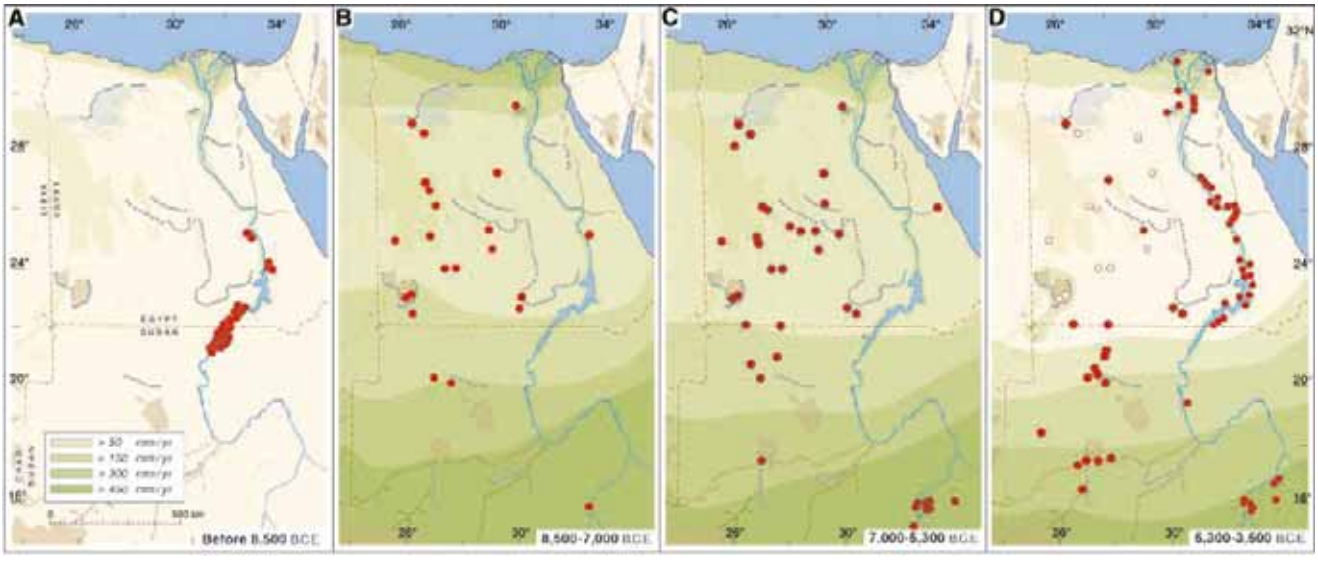

Fig. 3. Climate-controlled occupation in the Eastern Sahara during the main phases of the Holocene. Red dots indicate major occupation areas; white dots indicate isolated settlements in ecological refuges and episodic transhumance. Rainfall zones are delimited by best estimate isohyets on the basis of geological, archaeozoological, and archaeobotanical data. (**A**) During the Last Glacial Maximum and the terminal Pleistocene (20,000 to 8500 B.C.E.), the Saharan desert was void of any settlement outside of the Nile valley and extended about 400 km farther south than it does today. (**B**) With the abrupt arrival of monsoon rains at 8500 B.C.E., the hyper-arid desert was replaced by savannah-like environments and swiftly inhabited by prehistoric settlers. During the early Holocene humid optimum, the southern Sahara and the Nile valley apparently were too moist and hazardous for appreciable human occupation. (**C**) After 7000 B.C.E., human settlement became well established all over the Eastern Sahara, fostering the development of cattle pastoralism. (**D**) Retreating monsoonal rains caused the onset of desiccation of the Egyptian Sahara at 5300 B.C.E. Prehistoric populations were forced to the Nile valley or ecological refuges and forced to exodus into the Sudanese Sahara where rainfall and surface water were still sufficient. The return of full desert conditions all over Egypt at about 3500 B.C.E. coincided with the initial stages of pharaonic civilization in the Nile valley.

Eastern Sahara is the onset of semi-arid conditions in the north and semihumid conditions in the south at about 8500 B.C.E. Within only a few centuries, the desert margin shifted up to 800 km north to latitude 24°N, bringing monsoonal rainfall to most of the former desert. Taking into account the west-east gradient of decreasing humidity from the Atlantic Ocean to the Red Sea, this process apparently applied to the entire Sahara.

This fundamental climatic change from terminal Pleistocene hyper-arid desert conditions to savannah-type vegetation and the formation of lakes and temporary rivers resulted in the rapid dissemination of wild fauna and the swift reoccupation of the entire Eastern Sahara by prehistoric populations. Relatively stable humid conditions prevailed over approximately the next 3200 calendar years between 8500 and 5300 B.C.E. Abrupt drying events stated elsewhere in the Sahara may be explained by fading rainfall at a specific latitudinal position at a certain moment, or by dropping local groundwater.

The roughly parallel southward shift of monsoonal precipitation that set in at 5300 B.C.E. can be tracked through the following millennia by the discontinuance of the sedimentary record of aquatic deposits at decreasing latitudes. The geological archives in agreement with the archaeological evidence indicate a gradual desiccation and environmental deterioration of the Eastern Sahara, notwithstanding transitory climatic perturbations that are a common feature of all desert margins. This rather linear process culminates in the present extremely arid conditions, which have not yet reached the extent of the terminal Pleistocene (fig. S2F).

The southward movement of human settlement implied substantial changes in the pattern of behavior and land use as response to regional environmental differences. Most of all, mobility was the key to survival; it has driven prehistoric societies from foraging to a multi-resource economy and specialized pastoralism. The final desiccation of the Egyptian Sahara also had an essential impact on the contemporaneous origin of the pharaonic civilization in the Nile valley. To this day, conflicts in sub-Saharan regions such as Darfur are rooted in environmental deterioration, aggravated by severe demographic growth and man-made desertification. The presented data and conclusions suggest that the climate-controlled desiccation and expansion of the Saharan desert since the mid-Holocene may ultimately be considered a motor of Africa's evolution up to modern times.

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Supporting Online Material

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Genome Sequence Diversity and Clues to the Evolution of Variola (Smallpox) Virus

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Comparative genomics of 45 epidemiologically varied variola virus isolates from the past 30 years of the smallpox era indicate low sequence diversity, suggesting that there is probably little difference in the isolates' functional gene content. Phylogenetic clustering inferred three clades coincident with their geographical origin and case-fatality rate; the latter implicated putative proteins that mediate viral virulence differences. Analysis of the viral linear DNA genome suggests that its evolution involved direct descent and DNA end-region recombination events. Knowing the sequences will help understand the viral proteome and improve diagnostic test precision, therapeutics, and systems for their assessment.

Before eradication was declared in 1980, the *Orthopoxvirus* (OPV) variola virus (VARV) caused from ~1 to 30% case-fatality rates (CFRs) of smallpox, a strictly human disease. The infection began with a prodrome of systemic aches and a fever that peaked in about a week. As the fever broke, an oropharyngeal enanthema developed, followed immediately by an exanthema, a skin rash constituting an end stage of centrifugally distributed virus-filled pustules that felt "shotty," as if each contained a

pellet (*1*). Infectious VARV materials are in two secure repositories authorized by the World Health Organization (WHO); one is at the U.S. Centers for Disease Control and Prevention (CDC), and the other is at Russia's State Research Center of Virology and Biotechnology (VECTOR) (*2*–*4*). Between the two repositories, there is little overlap of isolates. Because a low-risk–high-consequence threat of smallpox by terrorism exists (*5*), the WHO oversees biosafety level 4 live VARV research aimed at developing accurate diagnostic tests, therapeutics, and systems to assess these countermeasures, which have been advocated by the U.S. Institute of Medicine (*6*).

To advance the research effort, we undertook the present comparative genomics study to investigate features of the sequence diversity and evolutionary relationships of 45 temporally, geographically, and epidemiologically varied viral isolates (table S1). Most isolates date to the WHO Intensified Smallpox Eradication Programme (1967 to 1980), a campaign for eliminating endemic disease in Africa, Asia, and South America and preventing smallpox spread back into Europe and elsewhere (*1*). For this study, we sequenced 43 isolates, including the previously described BSH75_banu (*7*, *8*), which we resequenced, and sequenced cowpox virus CPXV-GER91, vaccinia virus VACV-AC2000, and Tatera gerbilpox virus TATV-DAH68 (table S1). We also used reported genome sequences of VARV IND67_mah and BRZ66_gar (*9*, *10*). Forty-four VARV isolates are at the CDC; IND67_mah is at VECTOR.

Many of the isolates are associated with a CFR, an epidemiological estimate that enables classification of outbreak patterns roughly as minor (mostly less than 1% CFR) or major (usually more than 10% CFR). CFRs are generally reasonable estimations of VARV innate virulence, considering that inconsistencies—including case-patient health, age, health care quality, and immune status—affected such calculations. Low-CFR outbreaks of mild smallpox known as "alastrim" first became obvious in the Americas at the turn of the 20th century, which was about when another minor form called "amaas" appeared in Africa (*1*). It was not until the 1960s that a VARV growth-ceiling temperature test (*11*) enabled phenotyping that differentiated alastrim virus from amaas and other VARVs, thereby validating that at least two subspecies existed.

In West Africa, outbreaks with age-adjusted intermediate CFRs were observed, which motivated attempts, with some success, to differentiate isolates into three classes by using the thermal test and other tests (*1*, *12*–*14*), but eradication ensued and taxonomic interest faded. Despite some discordance, CFRs are all that remain for understanding the innate virulence of different isolates, so we used CFRs to help us recognize putative encoded proteins that might mediate virulence differences between the isolates. The associated CFRs of some isolates are discussed in the supplemental text (*15*).

The VARV genome. VARV contains a linear genome DNA of ~186 kilobase pairs (kbps) with covalently closed ends adjoining small inverted terminal repeat (ITR) regions that flank the coding region sequences (CRS) (Fig. 1) (*7*, *8*, *16*, *17*). Depending on the isolate, software predictions indicate that the CRS of the 45 isolates contain 196 to 207 open reading frames (ORFs), using a low-end cutoff of ≥50 codons (tables S1 and S2), which indicates that the isolates have essentially the same functional gene content. Like all poxviruses, ORFs are closely spaced and nonoverlapping, and there is no mRNA splicing (*18*); however, unlike other poxviruses, VARV lacks ORFs in the ITR region (fig. S2) (*16*, *17*). Depending on the isolate, nearly 90% of predicted ORFs coincide with entire ORFs predicted for other OPVs, and the rest appear to be ≥50-codon segments of entire or partial ORFs predicted for other OPVs, mainly the archetype VACV (fig. S1, annotation).

Intragenomic sequence diversity. Most VARV DNA preparations show, at nucleotide positions ~14,000 and ~161,000 (Fig. 1), respectively, a different number of T and AT repeats (table S3), which suggests that DNA replication involves site-specific viral DNA polymerase stuttering or intragenomic recombination. The T variance might provide frame-shift modulation at a polylysine site encoded by ORF-16, a homolog of a VACV ORF for an intracellular kelchlike protein that somehow alters Ca^{++}-independent adhesion of infected cells to the extracellular matrix (*19*). Ubiquitination of a variable lysine site might diversify an antigenic determinant. The AT variance precedes ORF-192, a VACV-WR ORF homolog for an interleukin-1β binding protein. VACV mutants deficient in this protein induce fever in mice infected intranasally and are more virulent than the parental VACV (*20*). The variance might ensure an ORF-192 deficiency in VARV.

Intergenomic sequence diversity. To investigate intergenomic sequence diversity, we aligned the 45 VARV CRS into a multiple alignment. We calculated diversity (π) of nucleotide sites across the alignment by counting single-nucleotide polymorphisms (SNPs) in columns with no gaps, and to make maximal use of the alignment, we scored insertions-deletions (indels, i.e., gaps) of ≥1 base as a single polymorphism (*15*). The π values derive from the 990 possible pairs of the 45 VARV CRS and represent 1782 specific SNPs and 4812 specific indels. By sliding a window of a defined site length and site step size across the alignment, we plotted (π) midpoint values (Fig. 2A). The diversity distribution agrees with general observations that the central CRS of poxviruses mainly specify conserved proteins essential for virus replication and that the terminal CRS encode for more divergent proteins, including those modulating host range and virulence (*18*). To estimate the extent of diversity of one ORF from another in the genome, we also divided the π value of each ORF of the 45 VARVs by its average number of codons to obtain π^{rel} (fig. S1). In a

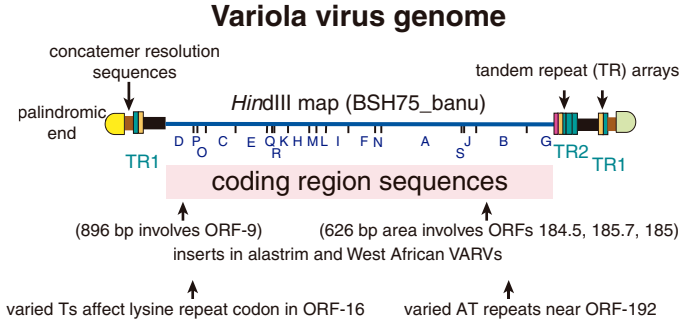

Fig. 1. VARV genome architecture. The VARV genome, depicted on the BSH75_banu *Hin*dIII map (*40*), is a linear ~186-kbp covalently closed DNA. The ~60-bp end loops (sequences not determined) are palindromes (light green or yellow) distal to concatemer resolution sequences (brown). Tandem repeat (TR) array units (aqua, orange, or magenta) flank the CRS (pink), which contain all the ORFs. TR arrays are within regions of inverted terminal repeats (ITRs) that contain no genes (fig. S2). TRs contain 69 to 70 bp units (aqua), partial units of 54 bp (orange) or 22 bp (magenta). The area has segments of nonrepeated sequences (black). The extra TR arrays (e.g., TR2) make the DNA topographically asymmetrical, although some isolates have a symmetrical appearance (fig. S2). Other areas indicated include those containing repeated Ts or ATs found in several isolates (table S3) and those showing gene-loss areas involving ORF-9 and ORFs 184 to 185 (fig. S3).

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sense, π^{rel} describes the extent of branching of a phylogram of an ORF.

To establish the diversity between any two isolates of interest, we separately summed the SNPs and the indels for each of the 990 VARV pairs of CRS (table S4). Relative to the size of the VARV genome, all pairs show low numbers, consistent with low diversity, which increases the likelihood that sequence-based detection methods will effectively identify a re-emergent VARV. To understand diversity further, we used sliding window plots of π midpoints of VARV pairs of interest, such as between isolates from the same outbreak or those that differ geographically or temporally (Fig. 2, B to G).

Because there is concern (*21*) that biotechnology enables construction of dangerous pathogens from genetic material of naturally occurring organisms, we plotted π midpoints and calculated the number of SNPs and indels that distinguish VARV from other OPVs, including isolates of monkeypox (MPXV), camelpox (CMLV), and gerbilpox (TATV) (Fig. 2, H to J). The results indicate that just a few thousand mutations by one of several rapid methods (*22*) could convert such OPV DNAs into VARV DNA. Recently, infectious VACV was recovered by rescuing its genome from a bacterial artificial chromosome containing its DNA (*23*). In theory, one could use sequences to synthesize long oligonucleotides (*24*) to reconstruct VARV DNA. Such rapidly advancing technology makes it important to understand sequence diversity and the proteome so as to develop and maintain countermeasures against malefic-use–created pathogens.

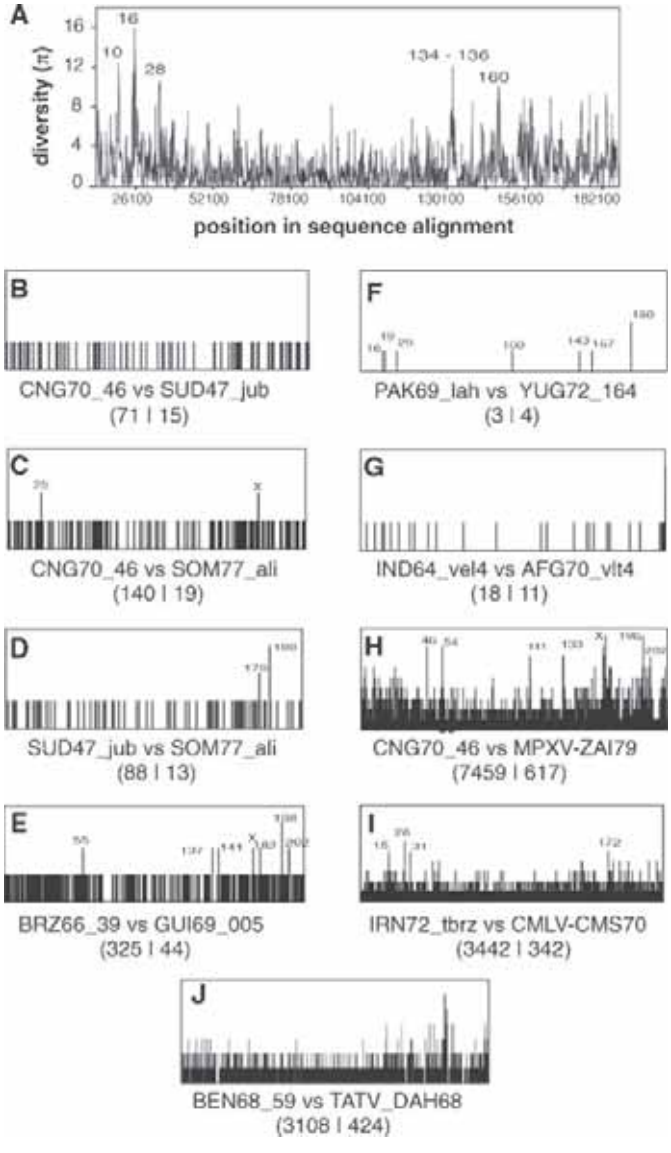

Fig. 2. Diversity of coding region sequences. (**A**) Polymorphic sites (*15*) determined by plotting diversity (π) midpoint values using a sliding window length of 200 sites moved in steps of 100 across an alignment of 45 VARV CRS. (**B** to **J**) Maps of π midpoint values [axis titles same as in (A)], using a window length of 12 sites moved in steps of 12, show loci separating viral pairs that differ by CFR, geographically, and/or temporally [SNP and indel values (table S3) are within parentheses]. (B) CNG70_46 (10% CFR) and SUD47_jub (<1% CFR). (C) CNG70_46 and SOM77_ali (0.4% CFR). (D) SUD47_jub and SOM77_ali. (E) BRZ66_39 (0.8% CFR) and GUI69_005 (8% CFR). (F) Pairing of isolates from the start and end of the 1969–1972 reintroduction of smallpox (16% CFR) into the Mideast and Yugoslavia (*1*) indicates a genetically very stable virus spread through hundreds of people. (G) Alignment of CRS of AFG70_vlt4 and IND64_vel4, which was isolated 5 years before the reintroduction, supports phenotypic and epidemiologic data implicating IND64_vel4 in the 1969 to 1972 Mideast smallpox resurgence (*41*). (H to J) Polymorphic sites that distinguish (H) CNG70_46 from Congo MPXV-ZAI79, (I) IRN72_tbrz from Iran CMLV-CMS70, and (J) BEN68_59 from TATV_DAH68. ORF number and X denote highly polymorphic sites within or outside ORFs, respectively.

The encoded proteome. Knowing the putative proteins and their diversity provides a framework for gaining insight into the actual proteome, mechanisms of antiviral drugs, systems to model the human response to VARV infection, and methods of immunological detection and treatment. Therefore, to compare the isolates, we selected one isolate as the comparator, namely BSH75_banu, and determined the amino acid sequence identity of each putative protein of each isolate to their BSH75_banu ortholog. The resultant data (table S2) were substantial, so we selected 10 of the most divergent VARVs (tables S4 and S5) to illustrate (fig. S1, left panel) that, with few exceptions, no more than about four amino acids in a protein distinguish the isolates. Consistent with π^{rel} and π, the main differences are in proteins encoded by terminal CRS.

Phylogenetic inference. To gain insight into VARV evolution, we aligned a 65-kbp section (Fig. 2A, nucleotide positions ~56,000 to ~121,000) of the mid-CRS of the 45 isolates and reconstructed an unrooted tree (Fig. 3A), which assumes the molecular clock hypothesis that mutation rates are equivalent along all branches of a tree (*15*, *25*). In addition, because poxviruses, including VARVs, can recombine readily (*26*, *27*) and recombination can confound tree reconstruction and an understanding of lineage, we reconstructed a phylogram (Fig. 3B) rooted using two outgroup OPVs, CMLV-CMS70 and TATV-DAH68, to rule out the molecular clock hypothesis (*25*); the phylogram also includes 18 VARVs representing the unrooted tree.

The VARV isolates in both trees branch into three main clades (strains taxonomically), which we denoted A to C (Fig. 3A) and which cluster according to the isolate's origin—West Africa, South America, or Asia, respectively. However, the clade-C Asian VARV cluster contains branches to subclusters, that is, derivatives; mainly, these are isolates (variants taxonomically) from non–West-African African countries, and these variants segregate into viral types of low- or mid-range CFR. In Fig. 3A, we show several CFRs [most from reference (*1*)]; the geographic grouping and strain clustering coincide with low-, mid-, and high-range CFR values. Atypically, the high-range (~30%) CFR IND67_mah stands out as more divergent than other isolates from India. The phylogram confirms epidemiologic data (*1*), which indicates that the German, Yugoslavian, and British isolates we sequenced are due to importations of high-CFR Asian-origin VARVs into Europe.

The phylogenetic method, which enabled systematically organizing by genotype what had been somewhat unsystematically collected isolates, supports late smallpox-era proposals based on comparative phenotyping tests that three distinct VARV classes exist—major, intermediate, and minor. The fact that three CFR classes coincide respectively with the

main genotypic clusters of viral strains and that intermediate and minor variant African types derive from main Asian VARV cluster probably caused many of the inconsistent results reported when researchers tried to correlate CFRs with phenotyping results (*1*, *12*, *14*, *28*).

Both trees (Fig. 3, A and B) show an ancestral node that is closer to and equidistant from clade A and clade B relative to clade C. Essentially, the branches connecting clades A, B, and C superimpose whether or not the tree is rooted, therefore the assumption of equal mutation rates seems nondistortive and tentatively acceptable. To look for further clues to better understand the VARV lineage, which in Newick format is either ((A,B)C) or ((B,A)C), thereby leaving the antecedence of A relative to B unresolved, we noted that the sequences of the indel areas (Fig. 1) in clade A are identical to their counterparts in clade-B. Moreover, we noted that clade C contains truncated versions of these indels. Together, the identity and a deletion within each indel area suggested an investigation for gene loss, which is generally clocklike (*29*), and for recombination, which can cause gene gain or gene loss and thereby markedly influence mutation rates governing branch lengths, which represent sequence distances (*25*).

Gene loss. The total number of nucleotides comprising clade-A and clade-B CRS are similar to each other and greater than the number constituting clade-C CRS (table S1); therefore, in its more distant separation, clade C lost more sequences, which suggests that gene loss played a role in VARV evolution. Additionally, in clade A and clade B the left-end indel includes ORF-9, a truncated version of the VACV-C9L–like ankyrin-repeat protein, which resembles the myxomatosis poxvirus protein M150R, a potential antiinflammatory component that subverts nuclear-factor-κB activation (*30*). Lastly, the clade-A and clade-B right-end indel causes formation of ORFs 184.5, 185, and 185.7, which encode hypothetical proteins (fig. S1 and table S2). These ORFs precede ORF-186, a homolog of VACV ORF-B12R, which encodes a serine-threonine kinase–like protein that does not phosphorylate in reactions that activate another VACV kinase (*31*).

To inspect the sequences containing these ORFs, we stratified the hierarchy of the CRS of the 45 VARVs by using a clade-A or a clade-B genome sequence to query a database containing the 45 VARV genome sequences in addition to genome sequences of selected other OPV strains. The stratifications show some sequence losses have not been at all random, particularly two that are specific for the left and right indel areas (Fig. 1) in clade C (fig. S3, A and B). Depending on which clade is the query sequence, as expected, the hierarchy agrees with the lineage ((A,B)C) or ((B,A)C). To focus on the left-end deletion, we queried the data with a 2-kbp segment of DNA that includes ORF-9 from a clade-A strain. The results (fig. S3C) show the deletion and possible gene loss within the clade-C VARVs, the lineage ((A,B)C), and the putative sources of the insert, possibly acquired by direct descent or recombination. Of sequenced OPVs, ORF-9 might be from TATV-DAH68, CPXV90_gri, CMLV-CMS, CMLV-M96, or the like. The right-end indel (fig. S3D) shows a deletion and possible gene loss within the clade-C VARVs and a lineage ((A,B)C). This indel might be from CPXV-GER91 (*32*) or the like, although TATV-DAH68 and CMLV are potential sources.

When we queried with ORF-184.5-186 sequences from a clade-A VARV, the lineage was ((A,B)C) and the origin of the ORFs appeared to be CPXV-GER91 (fig. S3E). Moreover, ORF-186 showed a distinct area of high sequence identity and another of low identity, which might be relevant to gain or loss of protein-kinase function.

Recombination. To assess whether the inserts were a result of recombination, we explored distance matrixes of multiply aligned CRS by using split-decomposition analysis (*15*), which produces reticulate treelike network graphs if a genetic sequence distance matrix evidences recombination. The algorithm yielded reticulate trees when the full CRS of VARVs representing each clade constituted the alignment input (fig. S4A); however, the network decomposed into a standard phylogram when the alignment was restricted to the mid-CRS (fig. S4A, insert). A split decomposition analysis with different OPV species produced a similar result (fig. S4B and insert). Together, the results infer recombination within the extremities of the CRS and thereby suggest that the end CRS have a different evolutionary history from the mid CRS, which further suggests that poxvirus phylograms might not be exact because of the influence of

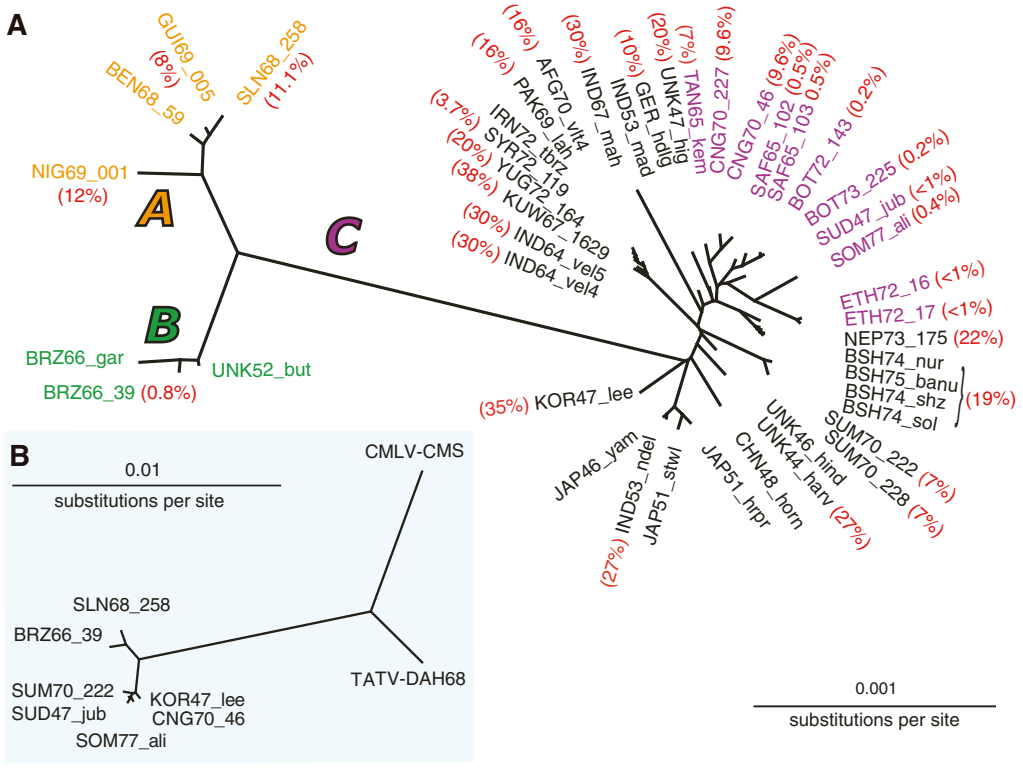

Fig. 3. Phylogenetic relationships. (**A**) An unrooted consensus phylogram from an alignment of 65 kbp of the mid-CRS of 45 VARVs reveals three high-level clades that represent clusters of isolates with origins in West Africa (clade A, orange), South America (clade B, green), and Asia (clade C, purple). The Asian clade contains a subgroup of non–West-African African variants (violet) that diverge into viral types of low- or midrange CFR. CFRs (red) associated with some isolates are indicated, and some of these are discussed in the supporting text (*15*). (**B**) A consensus tree rooted using CMLV-CMS70 and TATV-DAH68 mid-CRS aligned with a subset of VARVs representative of the tree in Fig. 3A.

recombination on mutation rates, and hence on tree branch lengths.

To investigate recombination further, we used sister-scanning assays (*15*) to quantify the significance (*Z* scores) of sequences being recombined. The algorithm slides a window of defined size across an alignment of two putative parents and a putative hybrid and essentially looks for patterns revealing sequence crossover. A significant likelihood of recombination was inferred by using a hypothetical alignment of the CRS of clade-A and clade-C VARVs as the parents and a clade-B VARV as the hybrid (fig. S5A). Similar results appeared using a clade-A VARV and CPXV-GER91 as the parents and a clade-B VARV as the hybrid (fig. S5B). Additional support for recombination became apparent by phylogenetic inference separately using the right-end indel recombined and flanking sequences (fig. S5C). Together the results in figures S3 to S5 suggest that evolution of the 45 VARVs involved gene gain by acquisition of the inserts, if indeed the ORFs represent genes and, if so, gene loss composed of the deletions in clade C compared with the other two clades.

Clade and encoded proteome relationships. Previous reports have compared genes of high-CFR members of clade C—BSH75_banu (CFR ~19%) and IND67_mah (CFR ~30%)—with each other and with genes of other OPVs, including VACV-COP and the low-CFR clade-A member BRZ66_gar (*7*, *8*, *10*, *33*–*36*). In the present study, we ascertained protein differences between low-range CFR clade-B and midrange CFR clade-A strains and between low- and midrange CFR clade-C variants from non–West-African Africa (Fig. 3A). The compilation used data from table S2, which contains the percentage identity between proteins of BSH75_banu and orthologs of the other VARVs. The amino acid sequence identity differences represent non-synonymous changes in codons that differentiate each ortholog from its correlate in BSH75_banu. The results reveal that a consensus of 67 putative ORFs distinguishes the group of the four clade-A strains from the group of the three clade-B strains and that a consensus of 15 putative ORFs distinguishes the mid- from the low-CFR groups of clade-C viral types from Africa (fig. S1, columns showing ORFs with non-synonymous nucleotide changes).

Discussion. In 1999, a U.S. Institute of Medicine report (*6*) advocated the scientific need for live VARV research to improve bioterrorism preparedness because infectious VARV samples might secretly exist (*5*). The report advised research to develop safe, efficacious antiviral drugs and a system for their assessment, and sequencing to refine the accuracy of VARV diagnostic tests and possibly provide additional scientific advances.

The low sequence diversity that we report here for a selection of isolates is reassuring and important from a biodefense perspective, because it suggests a high precision of differentiating VARVs if tracking single- or multi-source outbreaks. The ability to track the virus accurately might be a deterrent in its own right. The low diversity should also aid development of targeted, efficacious antiviral drugs and resolution of the actual proteome to help unravel the mechanism of action of the drug during infection of cell cultures or a model system. The low diversity of CRS indicates that the functional genome is not greatly varied, which improves the odds of understanding the proteome and reproducing smallpox in a system to assess countermeasures.

Treating the entire CRS as a seamless evolutionary unit oversimplifies reality. A main effecter of diversity in a population is natural selection, which causes genes within a genome to mutate. The relative diversity of each ORF (fig. S1, π^{rel}) and the percentage amino acid sequence identity maps (fig. S1, left panel) reveal that the proteins encoded within the terminal CRS vary the most, which indicates that selection pressure generally targeted these proteins the most. The pressure on the terminal regions of the proteome causing deletion and interruption probably drove the antecedent to become a devastating human pathogen.

Regarding the antecedent of the presently known VARVs, the OPVs most closely related to VARV are TATV_DAH68 (Fig. 2J) from a gerbil in Dahomey (Benin), Africa, and CMLV. The first credible physical evidence of smallpox is pockmarked Egyptian mummies that date to about 1500 BC (*1*). Given that there are missing links in the OPV lineage because the archived isolates are contemporary specimens and that there are various evolutionary possibilities, it is nevertheless tantalizing to speculate that gerbils and/or the sparse human population of ancient West Africa somehow spread smallpox into the more densely populated Fertile Crescent, perhaps when the Sudan-Sahel region across Africa was less arid. In addition, the appearance of alastrin might be related to the 18th-century slave trade of West African Yorubas tribesmen into Brazil.

Gene loss in general correlates with time (*29*), the inference being that if the ORFs in the indel areas represent real genes, then the encoded proteins might have been under negative selection, probably more by the human population in Asia than in Africa, as suggested by the often higher virulence of Asian VARVs in clade C (*25*, *29*). Increasing virulence reduces spread of a pathogen because, to the detriment of its own population, it kills off or maims the host population. Compared with the less virulent VARVs in clades A and B, the ORF-9 in clade C has a deletion, but deletions in the ortholog M150R are attenuating (*30*), which would constitute positive selection. However, there are examples like the interleukin-1β binding protein, which, if deleted, increases virulence of VACV (*20*).

Our study provides evidence for terminal region intergenomic recombination possibly within the species and with other OPVs (figs. S3 to S5). Considering the genome architecture (Fig. 1), one aspect of recombination that appears to be specific to VARV is the lack of intragenomic transposition in which genes from one end of the DNA appear duplicated and inverted at the opposite end of the DNA, probably through recombination events during replication, as described for other OPVs (*37*–*39*). We noted above that the production of site-specific, variable Ts and ATs might be due to DNA polymerase stuttering or intragenomic recombination. It is unknown whether recombination events in relation to the varied T and AT sites and the lack of VARV transposition have anything to do with each other.

Comparison of the amino acid percents identity between BSH75_banu and other VARV proteins (table S2) invited a determination of which proteins might influence virulence differences between low- and midrange CFR strains from clade A and clade B, respectively, and between clade-C African variant viral types of mid- or low-range CFR (fig. S1, candidate ORFs involved in virulence change). The ORFs of two clade-C Asian VARVs and the ORFs of these viruses and a clade-B virus have been compared previously (*10*, *33*). It is tempting to propose that the identified proteins alone modulate virulence; however, apart from these viral proteins, gene-control elements and yet undefined and undiscovered components could alter VARV innate virulence.

Our search for clues into the evolution of VARV is provocative, but it remains limited, partly because the repository represents a somewhat unsystematic collection. The present value of the viral stocks resides in their usefulness for understanding the VARV genome, the proteome, and the intracellular dynamics of infection, which will facilitate preparing for a natural, accidental, or deliberate release of VARV upon an unprotected world population.

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Supporting Online Material

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Materials and Methods
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REPORTS

Magnetic Fields in the Formation of Sun-Like Stars

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We report high-angular-resolution measurements of polarized dust emission toward the low-mass protostellar system NGC 1333 IRAS 4A. We show that in this system the observed magnetic field morphology is in agreement with the standard theoretical models of the formation of Sun-like stars in magnetized molecular clouds at scales of a few hundred astronomical units; gravity has overcome magnetic support, and the magnetic field traces a clear hourglass shape. The magnetic field is substantially more important than turbulence in the evolution of the system, and the initial misalignment of the magnetic and spin axes may have been important in the formation of the binary system.

Magnetic fields are believed to play a crucial role in the formation of stars (*1*, *2*). In the standard model of isolated low-mass-star formation (*3*, *4*), magnetized molecular clouds that are magnetically supported against gravitational collapse (or "subcritical") are expected to slowly form dense molecular cores through ambipolar diffusion. The neutral particles are only weakly coupled to the ions, which couple to the magnetic fields, and can drift toward the center of the cloud. The increasing central mass eventually overcomes the magnetic support and the now supercritical core collapses gravitationally. In this collapse phase, the initially uniform magnetic field is warped and strengthened in the core. The magnetic field is expected to assume an hourglass shape, with an accretion disk formed at the central "pinch" in the field, which corresponds, for an initial cloud size on the order of 10^4 to 10^5 astronomical units (AU), to scales of about 100 AU. At large scales, where the contraction effect is small the magnetic field lines are essentially straight. In this axisymmetric scenario, the process of magnetic braking, which forces the core to rotate at the same angular speed as the envelope, prevents fragmentation of the collapsing core and the formation of multiple stellar systems. However, models with nonaxisymmetric perturbations show that fragmentation can occur on a broad range of scales ($\leq$100 to 10^4 AU), depending on the initial conditions, such as magnetic field strength, rotation rate, and cloud mass–to–Jeans mass ratio (*5*).

The polarization of dust continuum emission provides an opportunity to examine the magnetic field configuration in star-forming regions (*6*). Aspherical spinning dust particles preferentially align themselves with the rotational axis (minor axis) parallel to the direction of the magnetic field. The emission from such grains is partially linearly polarized, with the observed polarization angle perpendicular to the direction of the magnetic field.

NGC 1333 IRAS 4A is a well-studied binary protostellar system in the Perseus molecular cloud complex (*7*). The two protostars, IRAS 4A1 and A2, are associated with molecular outflows directed roughly north-south (*8*). The distance to this cloud complex from Earth is thought to be between 220 and 350 parsecs (pc) (*9*); we adopt the value of 300 pc here. The Perseus complex is an active star-forming region with around 20 young stellar objects within a projected radius of 4×10^4 AU from IRAS 4A. Early polarimetric observations of IRAS 4A (*10*, *11*) did not have enough angular resolution to be able to examine the spatial scales relevant to the putative hourglass. Yet, in the highest angular resolution observations to date, polarimetry at 230 GHz with the Berkeley-Illinois-Maryland Association (BIMA) array showed hints of an hourglass shape in the magnetic field on 3.5″ (1000 AU) scales (*12*).

The Submillimeter Array (SMA) is the first imaging submillimeter interferometer (*13*, *14*), providing arcsecond angular resolution and good continuum sensitivity at frequencies that are higher than those currently observable with any other radio interferometer. We used the SMA to observe NGC 1333 IRAS 4A at 345 GHz at an angular resolution of 1.56″ $\times$ 0.99″ [with a position angle (PA) of 85°]. Our data resolve the continuum peaks of IRAS 4A1 and

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4A2, which we find to be separated by 400 AU (1.8″) at a PA of 130° (Fig. 1), as previously observed at lower frequencies at an angular resolution of ~0.6″ (*15*). Using the SMA polarimetry system (*16*), we are able to examine the magnetic field at 360 AU resolution and we find a clear "pinched" morphology (Fig. 1C) around this protostellar system. This provides a direct confirmation of the magnetic field configuration at the few-hundred–AU scale predicted by the standard theory of low-mass-star formation (*3*, *4*). Moreover, the detection of hourglass morphology even in this complex region suggests that the models of isolated star formation may apply even when the initial conditions are much less idealized than is normally assumed. Hints of magnetic field hourglass shape have also been reported in high-mass-star–forming regions such as NGC 2024 (*17*) and more clearly but at much larger scales (~0.5 pc) toward OMC-1 (*18*).

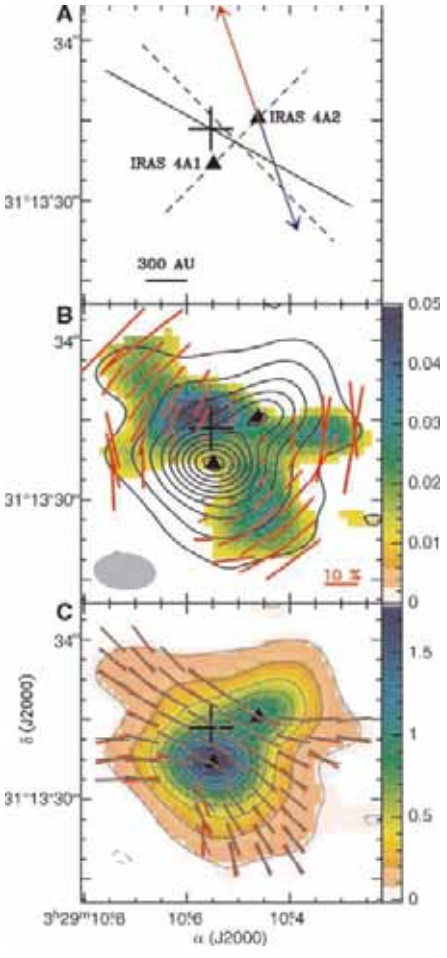

Fig. 1. (**A**) Sketch of the axis directions: red/blue arrows show the direction of the redshifted/blueshifted lobes of the molecular outflow, probably driven by IRAS 4B (*8*); solid lines show the main axis of the magnetic field; and dashed lines show the envelope axes. The solid triangles show the positions of IRAS 4A1 and 4A2. The cross shows the center of the magnetic field symmetry. (**B**) Contour map of the 877-μm dust emission (Stokes I) superposed with the color image of the polarized flux intensity. Red vectors indicate that length is proportional to fractional polarization, and the direction is the position angle of linear polarization. Contour levels are 1, 3, 6, 9,…30 × 65 mJy per beam. The synthesized beam is shown in the bottom left corner. (**C**) Contour and image map of the dust emission. Red bars show the measured magnetic field vectors. Gray bars correspond to the best-fit parabolic magnetic field model. The fit parameters are the position angle of the magnetic field axis $\theta_{PA} = 61° \pm 6°$; the center of symmetry of the magnetic field α_0(J2000) = 3 h 29 m 10.55 s ± 0.06 s and δ_0(J2000) = 31°13′31.8″ ± 0.4″; and $C = 0.12 \pm 0.06$ for the parabolic form $y = g + gCx^2$, where the x is the distance along the magnetic field axis of symmetry from the center of symmetry.

With the array configuration and frequency used, these SMA observations are not sensitive to dust emission on scales larger than 10″ or 3000 AU, where models of magnetized collapsing clouds expect the magnetic field to be uniform. Therefore, the magnetic field has been modeled by a family of parabolic functions using a χ^2 analysis. We find that the center of symmetry of the magnetic field coincides within the measured uncertainty, ~0.6″, with the center of the two cores. The position angle of the magnetic field axis, ≈61°, is roughly similar to the orientation of the magnetic field on larger scales around NGC 1333 (*21*). From Fig. 1C, we can see that across most of this region there is a remarkably accurate correspondence between the measured magnetic field vectors and the modeled parabolic magnetic field lines. However, there are some discrepancies southeast of the center, where the measured field seems to systematically deviate from the fitted model. The observed dispersion (Fig. 2), $\delta\theta_{obs}$, is made up of contributions from the measurement uncertainty of the polarization angle σ_θ and the intrinsic dispersion $\delta\theta_{int}$, according to the equation (*22*) $\delta\theta_{obs} = (\delta\theta_{int}^2 + \sigma_\theta^2)^{1/2}$. The observed dispersion ($\delta\theta_{obs}$) in the residuals is 8.0 ± 0.9°, whereas the measurement uncertainty of the polarization angle (σ_θ) is 6.2 ± 0.3°. Therefore, the intrinsic dispersion is $\delta\theta_{int} = 5.1 \pm 1.4°$. This estimate of the intrinsic dispersion should be regarded as an upper limit because the parabolic function is just a first approximation of the true magnetic field morphology.

If we assume that the dispersion in polarization angles is a consequence of the perturbation by Alfvén waves or turbulence in the field lines, then the strength of the magnetic field projected in the plane of the sky (B_{pos}) can be determined from the equation $B_{pos} = Q\ (\delta v_{los}/\delta\phi)(4\pi\rho)^{1/2}$, where ρ is the average mass density; δv_{los} is the line-of-sight velocity dispersion; and $\delta\phi$ is the dispersion in angular deviations of the field lines, which is the same as $\delta\theta_{int}$ calculated above (*23*). Q is a dimensionless parameter that depends on the cloud structure [$Q = 1$ corresponds to the original equation of Chandrasekhar and Fermi (*24*)]. Simulations of turbulent clouds suggest that $Q \approx 0.50$ (*25*), which is the value adopted. Using the value of the volume density derived from our data, $n(H_2) = 4.3 \times 10^7$ cm^{-3}, and the line width (corrected for the kinematical contribution) given by (*26*), $\delta v_{los} \approx 0.2$ km s^{-1}, we calculate the

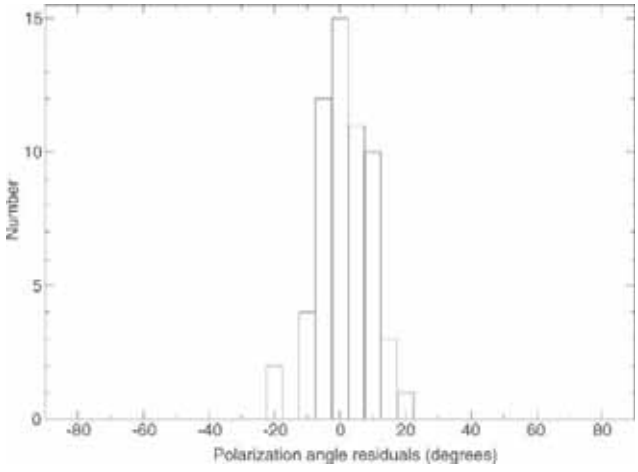

Fig. 2. Histogram of the polarization angle residuals for the best parabolic magnetic field model, shown in Fig. 1. The mean and the standard deviation of the polarization angle residuals are −1.1° and 8.0°, respectively.

magnetic field strength in the plane of the sky to be $B_{pos} \approx 5.0\ d_{300}^{-1/2}$ mG.

With this estimate of the magnetic field strength, we can compare the properties of NGC1333 IRAS4A derived from our observations to the theoretical predictions. The key parameter that determines whether magnetic fields provide support against gravitational collapse is the mass–to–magnetic flux ratio. Using the formula of (*27*), we find that the mass–to–magnetic flux ratio is $\approx 1.7\ d_{300}^{1/2}$ times the critical value for collapse. Uncertainties persist because of the neglect of the protostellar mass and the use of the plane-of-sky component of the magnetic field, which respectively increase and decrease this ratio. The estimated mass–to–magnetic flux ratio implies that the region traced by the SMA is slightly supercritical, which is what the theoretical models predict for the observed scales (*4*). This is further supported by the detection of observational signatures of infall motions (*26*).

These data also show that the magnetic energy dominates the turbulent energy in this source. This is demonstrated through β_{turb}, the square of the ratio of the turbulent line width σ_{turb} to the Alfvén speed V_A. From the expression given by (*17*), we find that $\beta_{turb} = 0.02$. Therefore, regardless of whether turbulence played a role in the initiation of the collapse of the parent cloud of NGC 1333 IRAS4A, it seems that at the observed stage of the star formation sequence in this region (the class 0 phase), magnetic fields dominate over turbulence as the key parameter to control the star formation process. Finally, the ratio of the magnetic tension to the gravity force, $f_{tension}/f_{gravity}$, demonstrates that gravitational forces are sufficient to cause the observed distortion in the magnetic field. This ratio is proportional to $B^2D^2/(R\ \rho\ M)$, where R is the radius of curvature of a given magnetic field line and D is the distance of the origin of this field line to the center of symmetry (*18*). From the south easternmost-modeled line of Fig. 1, we can estimate $R \approx 2.5''$ and $D \approx 1.6''$. Using these numbers and the mass derived from the dust emission, we obtain $f_{tension}/f_{gravity} \approx 0.20\ d_{300}^{-3}$. This value may be increased by a more accurate model for the magnetic field distribution (which would reduce the residuals and thereby increase the estimated magnetic field) or decreased by including the protostellar mass with the dust mass. Nevertheless, it is clear that the two forces are of similar order, as required.

The axis normal to the dusty envelope (44°) lies between the magnetic field axis (61°) and the main outflow axis (19°) (*8*). This suggests that when the collapse began, the spin and magnetic axes were not aligned. Could this misalignment be related to the observed formation of a binary system in NGC 1333 IRAS 4A? Studies of collapse in rotating magnetized cores show that fragmentation occurs only in the rotation-dominated cases (when centrifugal forces dominate over magnetic forces) (*28*, *29*). However, in these cases, if the initial spin and magnetic field axes do not coincide, the resulting magnetic field direction is expected to be substantially different from its original orientation. This is contrary to the conditions in IRAS 4A, where the observed field direction is roughly similar to the larger-scale magnetic field (*19*). In addition, as a consequence of the misalignment, the magnetic field geometry is predicted to be considerably distorted from the hourglass shape we observe. The current morphology of this object may indicate that the initial magnetic and centrifugal forces were comparable in magnitude (*29*), allowing fragmentation without substantial rotational distortion of the field.

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A Long-Period, Violently Variable X-ray Source in a Young Supernova Remnant

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Observations with the Newton X-ray Multimirror Mission satellite show a strong periodic modulation at 6.67 ± 0.03 hours of the x-ray source at the center of the 2000-year-old supernova remnant RCW 103. No fast pulsations are visible. If genetically tied to the supernova remnant, the source could either be an x-ray binary, composed of a compact object and a low-mass star in an eccentric orbit, or an isolated neutron star. In the latter case, the combination of its age and period would indicate that it is a peculiar magnetar, dramatically slowed down, possibly by a supernova debris disc. Both scenarios require nonstandard assumptions about the formation and evolution of compact objects in supernova explosions.

RCW 103 is a young (~2000 years) shell-type supernova remnant (SNR), with an x-ray point source very close to its center. Since its discovery (*1*), this source (1E161348-5055, hereafter 1E), characterized by unpulsed, soft x-ray emission and no radio or optical counterpart (*2*), has been considered a candidate neutron star (NS), obviously young, assuming that it was born in the same supernova (SN) event that originated the surrounding SNR. Subsequent x-ray observations have shown 1E to have a peculiar temporal behavior, with orders-of-magnitude secular flux variations (*3*, *4*). Variabilities and tentative periodicities for 1E have been proposed in a range from 1 to 6 hours (*5*–*7*). At the SNR's distance from Earth

(~3.3 kpc), 1E has a luminosity of 10^{33} to 10^{35} erg s^{-1}. Optical and infrared (optical/IR) observations point to an underluminous counterpart for this object: Three faint IR objects, with magnitude $H \sim 22$ to 23, lie in the Chandra error circle (*8*), but nothing is detected at visible wavelengths (magnitude $R > 25.6$, $I > 25$) (*6*, *9*).

Here we report results obtained during a long uninterrupted observation by the Newton X-ray Multimirror Mission (XMM-Newton) satellite on 23 August 2005, using the positive-negative (*10*) and metal-oxide semiconductor (MOS) (*11*) cameras of the European Photon Imaging Camera (EPIC) instrument. The total observing time was 89.2 kiloseconds (ks). Details on data analysis are given in the supporting online material (SOM). In the resulting MOS image of RCW 103 (Fig. 1A), 1E stands out at less than 20″ from the geometrical center of the remnant, itself ~9′ in diameter.

Our data show unambiguously that the source is periodic (Fig. 1B). The best estimate of the period (P) is 6.67 ± 0.03 hours (24.0 ± 0.1 ks). The flux modulation is large, with a pulsed fraction (PF) = 43.5 ± 1.8%. A search for fast periodicities was performed, with negative results. Periodicities with $P \geq 12$ ms and $PF \geq 10\%$ are excluded at the 99% confidence level.

As described in the SOM, time-averaged spectra for source and background were extracted for each EPIC detector in the 0.5- to 8-keV range. Single-component models do not yield acceptable results. The best fit is found for a double-component model consisting of a blackbody curve with temperature $kT \sim 0.5$ keV and emitting radius of ~600 m, contributing ~70% of the flux, complemented by either a second blackbody ($kT \sim 1$ keV) or a steep power law (photon index $\Gamma \sim 3$) (fig. S1 and table S1). The observed time-averaged flux is 1.7×10^{-12} erg cm^{-2} s^{-1} (0.5 to 8 keV).

The high XMM-Newton throughput and the long observing time yielded a total of 46,900 photons, which provides good enough statistics to allow us to perform phase-resolved spectroscopy. We find evidence for a definite hardening (a higher average photon energy) of the 0.5- to 8-keV spectrum at the peaks of the light curve and a softening at the troughs. Such a spectral evolution may be modeled by a higher temperature and larger emitting area of the dominant blackbody at the peak, coupled to a higher line-of-sight absorption (fig. S2).

The long-term time behavior of 1E before our 2005 observation was also studied and is summarized in Fig. 2. In August 2005, the source was clearly caught in a low state. We reanalyzed the 50-ks 2001 XMM data, when the source was in a higher state (~10^{-11} erg cm^{-2} s^{-1}). The periodicity seen in 2005 can be recognized (Fig. 2B, upper curve), albeit with a smaller PF (11.7 ± 1.4%). The period extracted from the 2001 data is 6.72 ± 0.08 hours (24.2 ± 0.3 ks), which is consistent with the 2005 value, with no evidence for a period variation. However, the source phenomenology is completely different. Apart from a factor of ~6 difference in the average flux value, the 2001 light curve has a much more complex substructure. The pulsed flux in 2001 is similar to the 2005 one, or ~2×10^{-12} erg cm^{-2} s^{-1}. The source time-averaged spectrum is significantly harder than in 2005, with a larger contribution from the high-energy component, as well as a larger absorption (fig. S3 and table S1).

The 6.67-hour periodicity reported here, as well as the long-term flux variability and complex spectral behavior, make 1E unusual among young compact objects still embedded in their SNR and make any interpretation very difficult.

The association of 1E and RCW 103 appears very robust, based on their perfect positional coincidence and on radio studies suggesting consistent distance estimates for the two objects (*12*). The chance alignment of a foreground object with the center of RCW 103 can be excluded on the basis of the optical data. Although an AM Her system (*13*) at 50 to 100 pc could show an x-ray phenomenology somewhat similar to the observed one, it would imply an optical/IR counterpart ~10 magnitudes brighter. Thus, we will assume that 1E was born together with its host SNR, which is 2000 years old (*14*).

Interpreting the 6.67 hours as an orbital period, we first explore a binary system hypothesis for 1E, featuring a compact object (either a NS or a black hole) born in the SN explosion and a faint star, for which existing optical/IR data (*6*, *8*, *9*) set stringent constraints. The colors and luminosity of the possible counterparts, assuming an interstellar reddening $A_V \sim 4.5$ (*12*), are compatible only with a M-class dwarf of ~0.4 solar mass ($M_\odot$) or smaller. 1E would thus be a low-mass x-ray binary (LMXB) that survived the SN event. However, 1E's phenomenology is very unusual for a LMXB. Its highly variable x-ray luminosity (~10^{33} to 10^{35} erg s^{-1}) is low, both compared to the peak luminosities of transient LMXBs (~10^{38} erg s^{-1}) and to the persistent LMXB output (10^{36} to 10^{37} erg s^{-1}). It is a luminosity similar to that of very faint x-ray transients (*15*), which are, however, very old systems (10^9 years).

Moreover, the pronounced orbital modulation and spectral phase variability reported here have never been observed in LMXBs. The same is true for 1E's long-term evolution, with its dramatic orbital modulation change and long outburst decay (Fig. 2).

Young age could be the explanation. Standard LMXBs are at least hundreds of millions of years older than 1E and have had enough time to evolve (*16*) to a phase in which the donor star, having filled its Roche lobe, supplies a large mass transfer toward the compact object, via an accretion disc, at a rate close to the Eddington limit. Conversely, in a very young, very-low-mass binary survivor of a SN explosion, a substantial orbital eccentricity is expected (*17*), with an important role in controlling any mass transfer within the system. For a dwarf star of mass M_d in the range of 0.2 to 0.4 $M_\odot$, an orbital eccentricity e of 0.5 to 0.2 would position the L1 point just above the dwarf star surface at periastron. Mass exchange would thus become possible within a narrow range of orbital phases, with a transit time of ~10 min from the donor through L1 toward the compact object. The transferred material, with its large angular momentum, would start settling in a disc. One also expects substantial orbital modulation in the fraction f of the dwarf star wind mass captured by the compact object. In the Bondi-Hoyle approach (*18*), $f \propto d^{-2} v_{rel}^{-4}$, where d is the

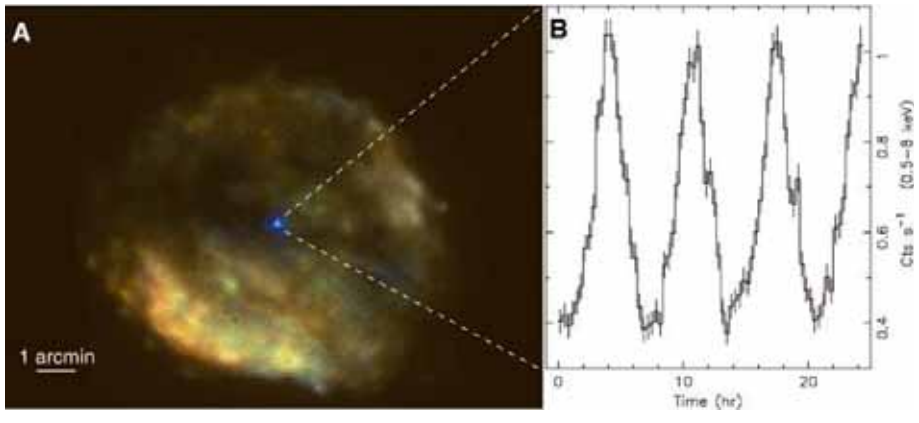

Fig. 1. (**A**) The young SNR RCW 103 and its central source 1E as observed in August 2005 by the EPIC MOS cameras onboard XMM-Newton. Photon energy is color-coded: Red corresponds to the energy range 0.5 to 0.9 keV, green to 0.9 to 1.7 keV, and blue to 1.7 to 8 keV. North is up, east is left. (**B**) Background-subtracted flux evolution of 1E in the 0.5- to 8-keV energy range, with its unambiguous 6.67-hour periodicity. Cts s^{-1}, observed counts per second.

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orbital separation and v_{rel} is the relative velocity between the dwarf star wind and the compact object. In 1E, f would vary by a factor ranging from 2.5 to 9 for an M_d of 0.4 to 0.2 $M_\odot$ and an e of 0.2 to 0.5, with a single peak during the descending part of the orbit, where the combination of velocities is most favorable (fig. S4).

A "double accretion" scenario could thus be at work. Wind accretion provides the bulk of the sharply peaked 6.67-hour modulation, while a disc controls 1E's long-term variations. Flux outbursts could be due to episodic mass ejections from the dwarf star and/or to disc instabilities, whereas dips in the light curve could be due to occultations by disc structures.

X-ray production remains the crucial point in such a binary model. If the compact object is a NS, accretion can occur if both the rotating dipole ("ejector") and the centrifugal ("propeller") barriers (*16*) can be overcome. The dynamical pressure of the infalling gas must exceed the pressure of the NS dipole radiation, at least down to a distance where corotation with the NS is slower than the keplerian velocity. This would imply that the 2000-year-old NS would have a very low magnetic field and/or a slow rotation period. Indeed, to produce via accretion a luminosity in the range observed for 1E, the above conditions imply (*19*) $P \sim (0.35 \text{ to } 2.5) B_{10}^{6/7}$ s, where B_{10} is the magnetic (B) field in units of 10^{10} G. These values are very peculiar if compared with the canonical picture of a standard 2000-year-old NS, having a B field of a few 10^{12} G and spinning at a few tens of milliseconds. On the other hand, if a black hole is present, accretion processes at the low rates implied by our young binary scenario are expected to be highly inefficient (*20*), and production of the observed luminosities could be problematic.

Faced with a highly nonstandard binary picture, we also consider an isolated-object scenario for 1E. We focus on NSs, because the periodical modulation rules against a black hole. The 6.67-hour periodicity could be related to the free precession of a fast-rotating NS, with the x-rays coming from a surface hot spot modulated at the precession period (*21*). However, we find no trace of the expected faster periodicity related to the star rotation. We cannot exclude a peculiar emission geometry, somewhat symmetrical with respect to the rotation axis, but find it an unlikely possibility. Similarly, a NS rotation period shorter than 12 ms seems unlikely, because some evidence of a synchrotron nebula or of nonthermal emission, due to the rotating dipole radiation, would be seen. A nonthermal x-ray output from $\sim 5 \times 10^{34}$ erg s^{-1} to $\sim 5 \times 10^{36}$ erg s^{-1} is expected (*22*) for a 12-ms pulsar with a 10^{12}-G B field. Moreover, any precession scenario would not explain the dramatic flux outbursts, together with the other long-term changes in the source phenomenology (Fig. 2).

Alternatively, 1E could be a normal isolated NS, slowly rotating at the 6.67-hour period. Some huge braking mechanism would have to be invoked to slow it down over 2000 years from its presumably much shorter birth period. To do this with the classical dipole-radiation pulsar mechanism requires the unheard-of, and probably unphysical, magnetic field value of $B \sim 10^{18}$ G. On the other hand, even if 1E were a "normal" NS with a birth period close to 6.67 hours, this would not account for its long-term x-ray flux variability.

1E could, instead, be a magnetar: a neutron star with an ultrahigh magnetic field of the order of 10^{15} G (*23*), now rotating at 6.67 hours. Indeed, all types of x-ray variabilities observed for 1E, as well as its luminosity and spectral shape, would be naturally explained in the magnetar frame. Magnetar candidates [namely, anomalous x-ray pulsars (AXPs) and soft gamma repeaters (SGRs)] show long-term variations in flux, spectrum, pulse shape, and *PF*. All magnetars, however, spin more than 1000 times faster than 1E, with periods well clustered in the 5- to 12-s range. The slowing-down mechanism obviously required for 1E could result from the transfer, through its rotating giant B field, of the star's angular momentum to the material of a hypothetical SN debris disc (a propeller effect). Our detailed calculations (*24*, *25*) show that a disc of 3×10^{-5} $M_\odot$ would have been enough to slow down, over 2000 years, a $B = 5 \times 10^{15}$ G magnetar, provided it was born with $P \gtrsim 300$ ms (fig. S5). Such a birth period is necessary for avoiding an early ejector phase, because the pressure of the radiation of the rotating dipole quickly pushes away any disc surrounding a fast magnetar. With a slower rotation at birth, the star instead retains its disc and immediately begins a very efficient loss of rotational energy. A birth period $\gtrsim 300$ ms is too long to fit into the most popular explanation (*26*) for the origin of the huge B fields of magnetars (a dynamo effect in the proto-NS, requiring a birth period of $\sim$1 ms). However, alternative high–B-field formation scenarios (such as compression of the progenitor field) have been proposed (*27*, *28*), based on possible evidence that not all magnetars are born as very fast rotators (*27*).

The recent discovery of a debris disk around an AXP (*29*) may support a "braked magnetar" picture for 1E, suggesting that at least some magnetars could be surrounded by fossil disks. AXPs and SGRs, as witnessed by their 5- to 12-s periods, did not experience an efficient propeller phase, possibly because of a shorter period at birth or strong gamma-ray bursting activity. If 1E is indeed a slow magnetar, this implies a totally new evolutionary channel for isolated NSs, one in which their spin history is dominated by SN debris. The fraction of NSs following such a channel should be small, however, considering the unusualness of 1E among compact objects associated with SNRs. Furthermore, as for standard AXPs and SGRs, it may be that such objects rapidly (in 10^5 years?) become unobservable. However, one could think of some compact objects not

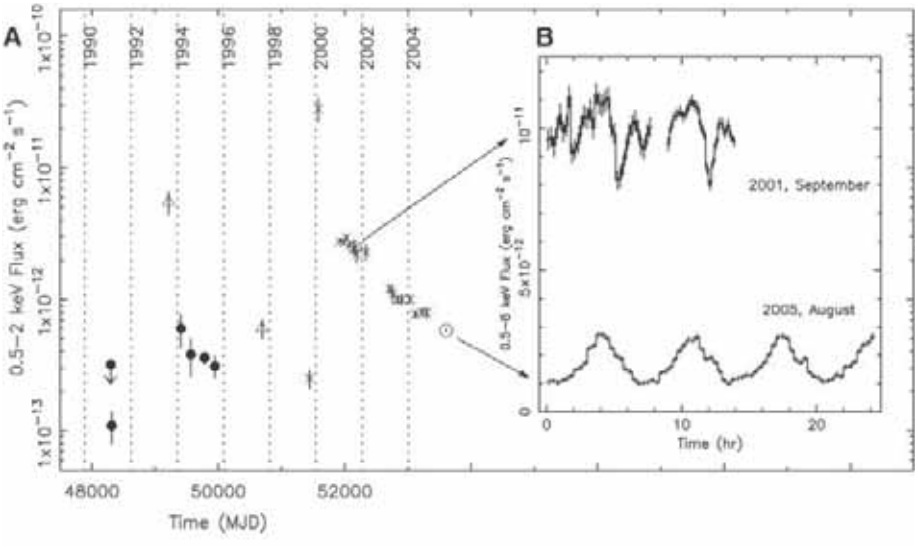

Fig. 2. A synoptic view of time variabilities of 1E. (**A**) Source secular flux evolution as derived from our analysis of the public Chandra observations (crosses) performed between 1999 and 2005 and two XMM-Newton ones (open circles) performed in 2001 and 2005. A large, two–order-of-magnitude outburst between 1999 and 2000 is followed by a continuous fading down to the level of our 2005 XMM observation. Historical measurements (*3*) with the Rosat (black solid circles) and ASCA (triangles) satellites are also included and show another episode of flux increase around the ASCA observation in 1993. Source outbursts could thus be recurrent on a several-year time scale. (**B**) Source flux variation over the 2001 (upper curve) and 2005 (lower curve) XMM-Newton observations, of 50 and 90 ks, respectively. Observation starting times have been aligned, but no folding has been performed. The 6.67-hour periodicity may be seen in the shorter 2001 observation, although with a smaller *PF* and a much more complex pulse shape.

showing now any pulsation, in spite of large observational efforts, such as sources associated with young SNRs (such as Cas A, VelaJr, and G347.3-0.4) (*6*).

Other scenarios could also be explored. We may consider a peculiar binary system in which the 6.67-hour periodicity reflects the spin period of the collapsed object (necessarily a NS), but in which the orbital period is much longer and undetected. As in the isolated NS case, the main difficulty here is to account for a huge braking of the NS rotation in 2000 years, unless it was born spinning at 6.67 hours. As in the case of 2S 0114+650 (*30*), a binary system featuring a 2.7-hour-period NS and a giant companion with an estimated age of $>10^7$ years, the only viable mechanism could be the propeller effect on the wind of the companion star. Following (*19*), we note that for plausible parameters of the accretion rate in the 1E system ($\dot{M} \sim 10^9$ to 10^{10} g s^{-1}), an equilibrium period of 6.67 hours could indeed result for NS *B* fields on the order of 10^{12} to 10^{13} G, but the overall NS spin-down process would require 10^8 to 10^{10} years. Assuming instead a magnetar *B* field of 5×10^{15} G and a higher (but still plausible) accretion rate of 10^{13} g s^{-1}, the braking would be much more efficient, but in any case >20,000 years would be required to reach a period similar to the observed one. Thus, such a picture seems untenable. One could postulate that 1E and RCW 103 were generated by two different SN explosions within the same binary system, originally composed of two high-mass stars. The first SN produced 1E (at least $\sim 10^5$ years ago, to allow for the fading of the resulting SNR) and did not disrupt the binary. The second produced RCW 103 ~2000 years ago but did not leave any visible compact object. 1E could have been slowed down over the lifetime of its companion star ($\sim 10^7$ years?), remaining in any case an active magnetar, as is required to explain its time behavior. Occam's razor argues against such an interpretation. For a scenario involving a magnetar, the braking of a young isolated object by SN debris seems the most plausible explanation.

Many more details remain to be explored regarding both 1E and RCW 103. Deeper and longer x-ray observations could detect fast pulsations, ruling out the slow rotator model proposed above. Observations during the source's high state could allow for phase-resolved spectroscopy, giving evidence of any intervening circumstellar occulting material. Optical/IR observations could yield the nature of any optical counterpart, to check, for example, on the presence of a disc. It would be also useful to carry out spectral studies of the diffuse remnant material. Although difficult, such studies could be crucial in understanding a SN event that, 2000 years ago, created either a compact object or a binary system so unusual in its physical properties.

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Supporting Online Material

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Hexagonal Mesoporous Germanium

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The blending of mesoporosity with the properties of semiconductors promises new types of multifunctional nanomaterials. It would be particularly interesting to combine the shape selectivity of a mesoporous oxide with the electronic and photonic characteristics of a useful semiconductor. We demonstrated the synthesis of a mesoporous germanium semiconductor using liquid-crystals-templated chemistry. The template removal was achieved by a two-step ion-exchange thermal procedure. This semiconductive mesoporous form of germanium possesses hexagonal pore ordering with very high surface area and exhibits strongly size-dependent optical properties as well as photoluminescence.

The physical and chemical properties of mesoporous (pore size from 20 to 500 Å) solids arise from a well-defined pore structure, high internal surface area, and their framework composition. Mesoporous silicates and transition metal oxides have been extensively studied for their adsorption, separation, catalytic, and magnetic applications (*1*–*3*). Mesoporous carbons, noble metals, and mesostructured organic-inorganic hybrid chalcogenides materials have also been reported (*4*–*12*). The pores of a mesostructured material may or may not be accessible. When the pores become accessible through template removal or otherwise, the system is then defined to be mesoporous. Similar to the silicates, the mesostructured chalcogenides exhibit long-range pore order; however, they have not been rendered porous, and in general attempts to remove the template from the pores result in framework decomposition. Mesoporous semiconductors with well-defined pore structure are relatively unknown materials. Recently, crystalline microporous (pore size < 20 Å) chalcogenides (*13*) and porous chalcogenide aerogels (*14*) that possess high porosity have been reported. Porous semiconducting frameworks could exhibit unique properties such as a combination of quantum-

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confinement effects, coupled electronic properties, high internal surface area, and size-selective absorption (*15*).

Germanium, like silicon, is an important semiconductor of broad fundamental and technological interest (*16*). We recently showed that a well-ordered cubic gyroid mesophase of all-germanium semiconductor (MSU-Ge-1) can be synthesized (*17*). This mesostructure could not be rendered porous, because attempts to remove the surfactant caused the framework to collapse, partly due to its very thin walls (~1 nm). Here, we describe a different synthetic strategy that leads to mesoporous germanium with very high internal surface area. This mesoporous germanium (MSU-Ge-2) exhibits a considerably blue-shifted energy gap relative to the bulk germanium as well as photoluminescence.

A family of mesoporous all-germanium semiconductors was prepared with the use of templating Ge polymerization by liquid-crystalline phase according to the metathesis reaction of Eq. 1. Namely, linking Ge_9^{4-} clusters (Fig. 1 inset) in formamide solution with germanium tetrachloride ($GeCl_4$) in the presence of self-assembled surfactant resulted in formation of a well-ordered mesostructure. Presumably, the surfactant creates an organized lyotropic liquid crystalline phase to template the inorganic Ge framework (*1*):

$$\text{R-NMe}_2(\text{CH}_2\text{CH}_2\text{OH})\text{Br} + \text{K}_4\text{Ge}_9 + \text{GeCl}_4 \xrightarrow{\text{formamide}} [\text{R-NMe}_2(\text{CH}_2\text{CH}_2\text{OH})]_{0.8}\text{Ge}_{10} + \text{KCl} + \text{KBr}$$

MSU-Ge-2 (1)

where R equals C_nH_{2n+1} and *n* equals 18, 20, or 22.

While this manuscript was under review, the preparation of a mesoporous germanium with hexagonal pore symmetry was reported (*18*). This material was prepared from the oxidative coupling of polymeric $^1/_\infty(Ge_9)^{2-}$ species without a linking ion.

The chemical composition of the product, MSU-Ge-2 with *N*-eicosane-*N,N*-dimethyl-*N*-(2-hydroxyethyl) ammonium bromide (EDMHEAB) surfactant, is $(EDMHEAB)_{0.8}Ge_{10}$, as determined by elemental C, H, and N and thermogravimetric analysis (TGA) (*18*). The purity of the inorganic framework was confirmed by energy dispersive spectroscopy (EDS), where only Ge element was observed (Fig. 1A). Potassium or halide (Cl or Br) ions were not detected, suggesting a complete metathesis of all Ge-Cl bonds.

Surfactant removal was achieved via a two-step ion-exchange thermal approach. First, the surfactants were replaced with ammonium nitrate (NH_4NO_3). The successful incorporation of NH_4^+ cations inside the Ge framework was confirmed with elemental C, H, and N analysis (*19*) and Fourier-transform infrared (FT-IR) spectroscopy, which showed characteristic N-H absorption bands at ~1420 cm^{-1} (Fig. 1C, curve b). The subsequent removal of the NH_4^+ cations from the mesopores was achieved with mild heating to give hexagonal amorphous Ge with very high mesoporosity (*20*). After the thermal treatment to remove the NH_4^+ ions, FT-IR spectroscopy showed the presence of Ge-H_x stretching bands at 2141 and 2011 cm^{-1} without the characteristic N-H bending band (Fig. 1C, curve c). This suggests that the removal of NH_4^+ ions was in the form of volatile NH_3 molecules, whereas the H^+ ions transferred to the Ge framework to produce Ge–H bonds passivating the surface (*21*).

Thermal gravimetric analysis profiles of as-prepared MSU-Ge-2 material show a weight loss of 5.4% (weight/weight, w/w) below 210°C, which is due to the removal of physisorbed and possibly chemisorbed formamide. Between 210° and 580°C, a gradual weight loss of 28.2% (w/w) is observed, which is attributed to the decomposition of surfactant (Fig. 1B). The inorganic residue at 600°C of both as-prepared and template-removed materials was primarily crystalline Ge. The formation of elemental Ge supports the notion that the framework in MSU-Ge-2 is in fact reduced.

The long-range periodicity of the pore structure of as-prepared MSU-Ge-2 and template-removed material was confirmed with powder x-ray diffraction (XRD) and transmission electron microscopy (TEM). The XRD patterns exhibit a broad strong peak at low scattering angles ($2\theta < 3°$) and a very weak feature at $2\theta \sim 4°$, which, according to TEM, could be attributed to hexagonal *p6mm* symmetry (Fig. 1D). The *d* spacing of the first strong reflection gives a pore periodicity of ~4.8 and ~4.6 nm, respectively, for as-prepared MSU-Ge-2 (containing surfactant) and template-removed material (*22*). The absence of Bragg reflections at high angles ($2\theta > 10°$) in the XRD pattern indicates the aperiodic nature of the inorganic framework and the absence of crystalline Ge impurity.

TEM images and the corresponding fast-Fourier transform (FFT) patterns of as-prepared MSU-Ge-2 and template-removed mesoporous Ge are depicted in Fig. 2. The TEM and FFT analysis confirm the presence of the well-defined long-range hexagonal arrangement of the pore channels in the as-prepared material (Fig. 2, A and B). The images also confirm that the hexagonal pore ordering is retained even after NH_4^+ ion exchange and heat treatment of the sample (Fig. 2, C and D). TEM analysis indicates average pore-pore distances of ~4.0 and ~3.8 nm (*22*) and pore diameters of ~2.3 and ~2.0 nm, respectively. This gives a framework wall thickness of ~1.8 nm.

Clear evidence of the mesoporosity of MSU-Ge-2 after template removal was obtained

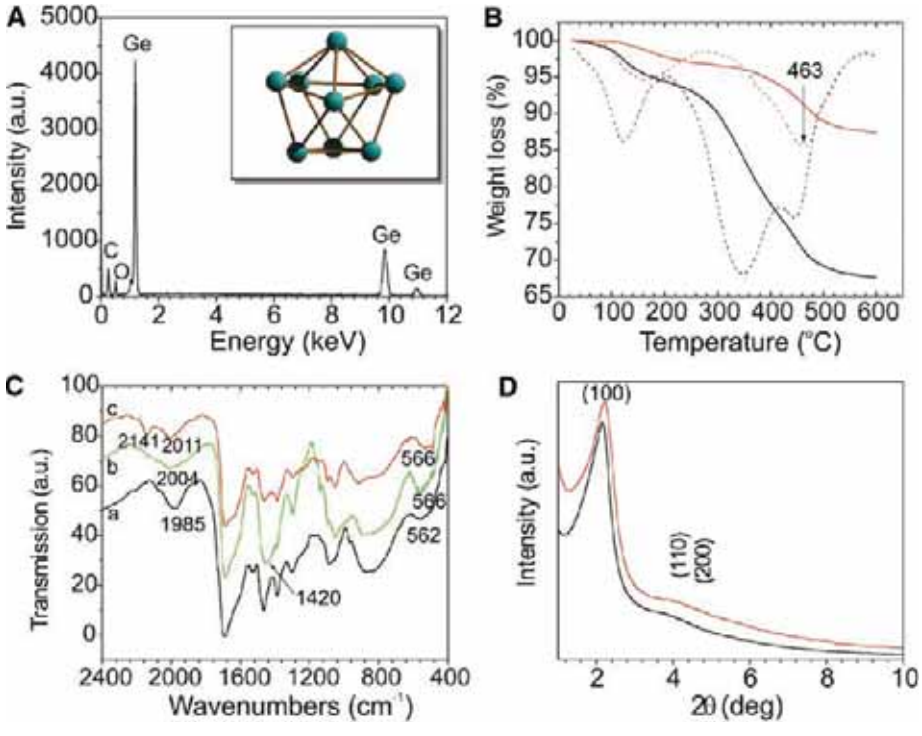

Fig. 1. (**A**) EDS spectrum of as-prepared MSU-Ge-2 semiconductor. The carbon and oxygen related peaks are attributed to the sample (surfactant) and the grid. (Inset) Molecular structure drawing of the deltahedral Ge_9^{4-} cluster (**B**) TGA profiles (solid lines) performed in nitrogen of as-prepared MSU-Ge-2 (black) and heat-treated NH_4^+-exchanged material (red). The temperatures of weight loss are indicated in the corresponding differential thermogravimetric (DTG) curves (dashed lines). (**C**) FT-IR spectra of (a) as-prepared MSU-Ge-2, (b) NH_4^+-exchanged, and (c) heat-treated NH_4^+-exchanged materials. The absorptions in the 1900 to 2250 cm^{-1} region are due to Ge–H bonds. (**D**) Low-angle powder XRD pattern of as-prepared MSU-Ge-2 (black) and heat-treated NH_4^+-exchanged mesoporous Ge (red).

with nitrogen physisorption measurement. The adsorption-desorption isotherms show a type IV adsorption branch associated with a well-defined capillary condensation step at $P/P_o \sim 0.13$, characteristic in uniform mesopores (Fig. 3A). The very small hysteresis between the adsorption and desorption branches (at $0.4 < P/P_o < 0.8$) is consistent with the parallel arrangement of uniform pore channels (pore size < 40 Å) (*23*). This is in agreement with the TEM images of Fig. 2. The adsorption data indicate a very high Brunauer-Emmett-Teller (BET) surface area of 363 $m^2\ g^{-1}$ and pore volume of 0.23 $cm^3\ g^{-1}$. Because the all-germanium mesostructure is much heavier than a corresponding silica one, this surface area is comparable to a silica with surface area of 1316 $m^2\ g^{-1}$ (*24*). The pore size distribution calculated by the Barrett-Joynes-Halenda (BJH) method from the adsorption branch is quite narrow, indicating a uniform pore structure with a pore diameter of ~2.1 nm (Fig. 3B). On the basis of the pore-pore spacing of ~4.0 nm determined from XRD, we obtained a framework wall thickness of ~1.9 nm, in good agreement with that observed from TEM. MSU-Ge-2 materials obtained with different surfactant chain lengths, $C_{18}H_{37}$- and $C_{22}H_{45}$-, also exhibited hexagonal pore symmetry and gave mesostructures with surface areas of 109 and 364 $m^2\ g^{-1}$, pore volumes of 0.09 and 0.40 $cm^3\ g^{-1}$, and narrow pore size distributions at ~1.7 and ~2.2 nm, respectively (fig. S1).

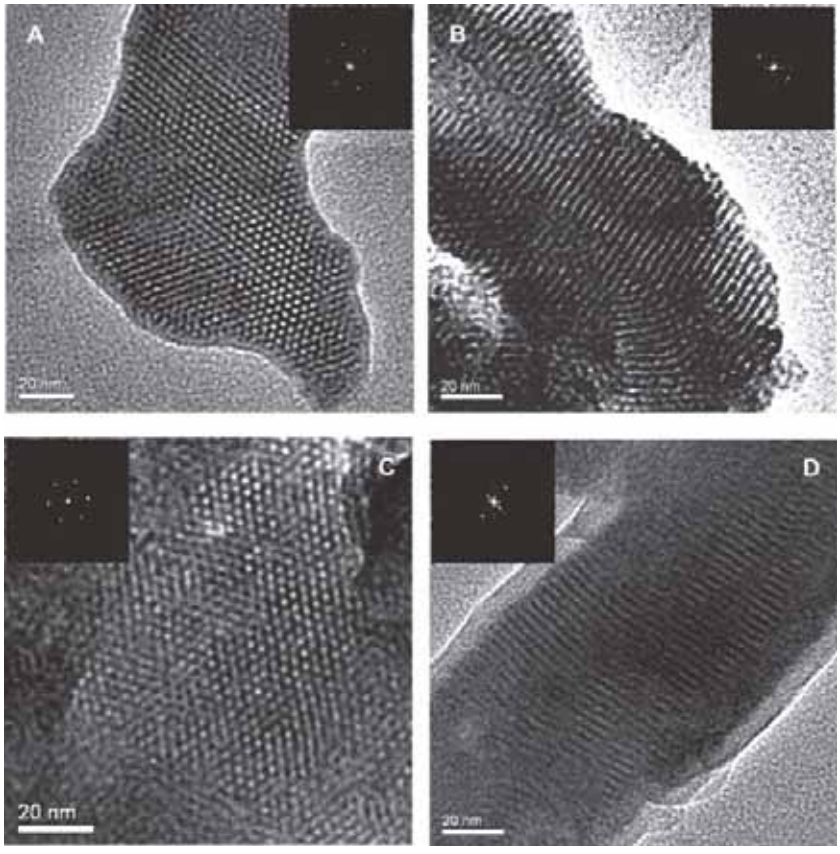

Fig. 2. TEM and (insets) FFT images. (**A** and **B**) As-prepared MSU-Ge-2. (**C** and **D**) Mesoporous MSU-Ge-2 obtained by heat-treating the NH_4^+-exchanged material. The images shows a periodic hexagonal array along the [100] direction (A and C) and a periodic array of parallel pore channels along the [110] direction (B and D).

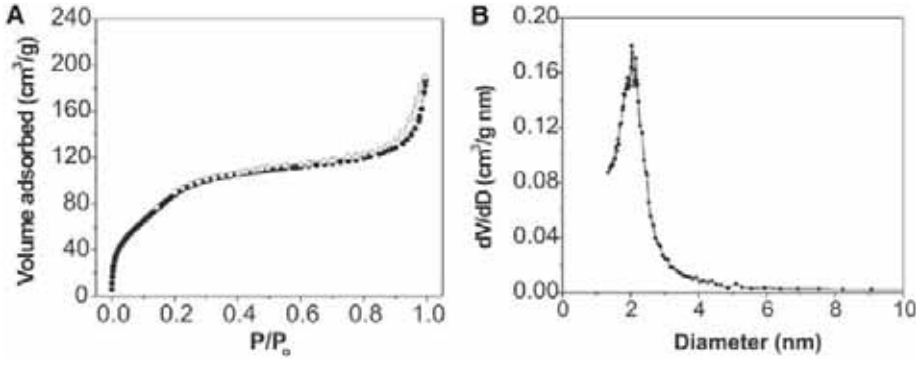

Fig. 3. (**A**) Nitrogen adsorption-desorption isotherms at 77 K of heat-treated NH_4^+-exchanged MSU-Ge-2 (solid circles, adsorption data; open circles, desorption data). The hysteresis observed at $P/P_o > 0.8$ is due to the interparticle voids between the agglomerated particles. (**B**) BJH pore size distribution calculated from the adsorption branch of the isotherm.

Pair distribution function (PDF) analysis was used to probe the local structure of the germanium framework (*25*). This technique allows the observation of first, second, and third neighbors in the structure. Figure 4A shows the PDF plots versus the interatomic distances in the as-prepared and mesoporous MSU-Ge-2 material, K_4Ge_9 precursor, and polycrystalline Ge. The PDFs of MSU-Ge-2 show strong interatomic correlation vectors at 2.4 and 4.1 Å, corresponding to Ge-Ge nearest neighbor and Ge···Ge next nearest neighbor distances, respectively. The well-resolved small peak at 5.8 Å in the template-removed MSU-Ge-2 sample suggests better ordering in the framework after surfactant removal. The lack of atomic pair correlation vectors at distances beyond 6 Å indicates a short-range order of -Ge_9- clusters in the mesoporous framework. To detect whether the -Ge_9- clusters were present in the framework, we also determined the PDFs of the K_4Ge_9 precursor and the crystalline Ge for comparison. As indicated in Fig. 4A, up to 6 Å the PDF profile of MSU-Ge-2 is more similar to that of K_4Ge_9 than to that of crystalline or amorphous Ge (*25*). The PDF of K_4Ge_9 shows characteristic peaks at 2.5 and 4.0 Å, which correspond to Ge-Ge first neighbors and Ge···Ge second neighbors inside the deltahedral $(Ge_9)^{4-}$ structure, respectively. The similarity of the PDF profiles between MSU-Ge-2 and K_4Ge_9 implies that the -Ge_9- cluster remains intact in the inorganic framework of the former. Further evidence for the existence of -Ge_9- cluster inside the MSU-Ge-2 framework was obtained by measuring the relative probability between the first Ge-Ge and the second Ge···Ge neighbor interatomic vectors, determined by the ratio of integrals of the first and second peaks in the PDF. For both as-prepared and mesoporous MSU-Ge-2, the ratios of 1.60 and 1.51, correspondingly, are close to the deltahedral $(Ge_9)^{4-}$ ratio of 1.46 and are very different from the value of 0.34 obtained from the local tetrahedral geometry of Ge atoms in bulk germanium.

The oxidation state of Ge in MSU-Ge-2 was probed with x-ray photoelectron spectroscopy (XPS). The XPS spectrum showed Ge 3d peaks with binding energy at 29.85 and 31.9 eV (Fig. 4B). This suggests at least two sites in the structure, one with low valence for Ge atoms, probably from the Ge_9 clusters, and a higher valence site possibly due to the Ge linking

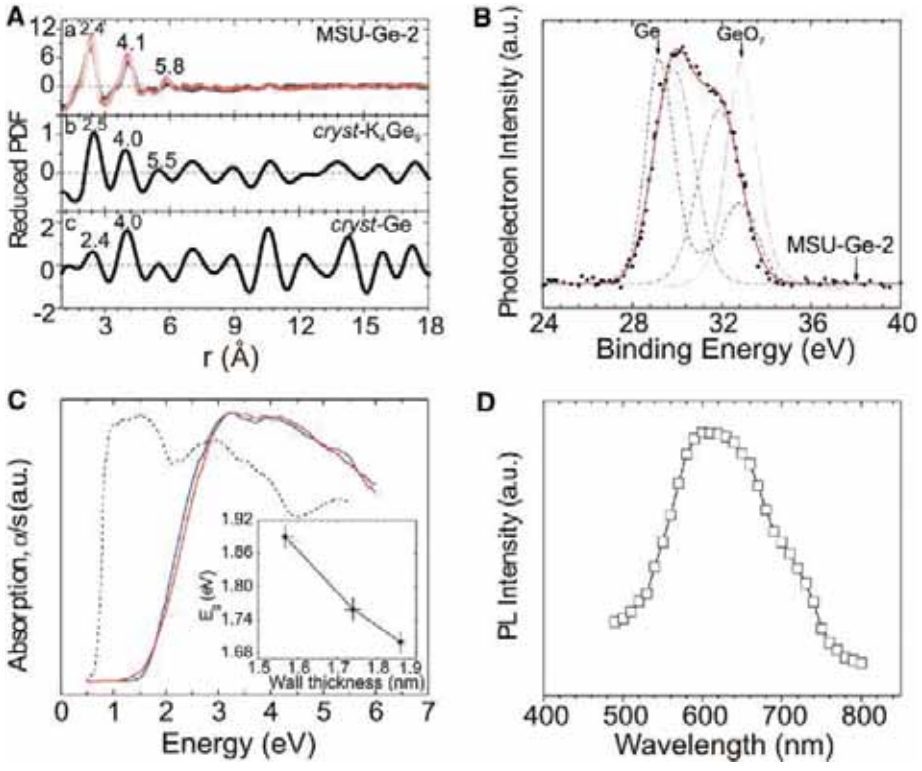

Fig. 4. (**A**) Reduced atomic pair distribution function $G(r)$ of (a) as-prepared MSU-Ge-2 (black) and heat-treated NH_4^+-exchanged mesoporous material (red), (b) crystalline K_4Ge_9, and (c) crystalline Ge. (**B**) XPS spectra of MSU-Ge-2 (solid circles), element Ge, and GeO_2 solids (dashed lines) for the Ge 3d signal. The peaks positions are from curve fitting the experimental data. (**C**) Optical absorption spectra of as-prepared (blue) and mesoporous MSU-Ge-2 (red) and bulk polycrystalline Ge (dashed line). (Inset) Size dependence of the energy band gap of MSU-Ge-2 as a function of framework wall thickness. Adjusting the reaction conditions of the MSU-Ge-2, the Ge-network demonstrates control of the wall thickness, which is reflected in the size of the energy band gap. (**D**) Room temperature photoluminescence spectrum of as-prepared MSU-Ge-2 with excitation wavelength at 240 nm. a.u., arbitrary units.

atoms. The Ge 3d spectra obtained from polycrystalline Ge (29.2 eV) and GeO_2 (33.9 eV) are very different from those of MSU-Ge-2, consistent with the fact that the latter is not a mixture of Ge and GeO_2 phases.

The optical absorption spectrum of as-prepared MSU-Ge-2 semiconductor shows a well-defined energy gap transition at 1.70 eV. This band gap is preserved in mesoporous material after surfactant removal (1.71 eV) (Fig. 4C). These energy gaps are much wider than that of crystalline or amorphous bulk Ge (0.66 eV). The enormous blue shift in energy gap compared with that of bulk Ge is due to the substantial dimensional reduction of wall thickness (~19 Å) in MSU-Ge-2 and is reminiscent of the quantum-confinement-induced blue shifts in energy gap observed in Ge nanocrystals (*26*). We found that, by adjusting the reaction conditions during the synthesis of MSU-Ge-2, the pore wall thickness can be varied from ~1.9 to ~1.6 nm. Evidence for this was obtained from N_2 sorption and XRD experiments (fig. S2). We also found that the energy gap decreases with an increase of wall thickness, which is consistent with a quantum confinement effect exhibited by the mesoporous framework (Fig. 4C inset). MSU-Ge-2 also showed photoluminescence (PL) at ~610 nm at room temperature when excited by photons with high energy (Fig. 4D). The origin of this photoluminescence is not yet understood.

The synthetic chemistry using Group IV Zintl clusters and their possibility for doping using different linker centers can lead to a variety of well-ordered mesoporous semiconductors. Furthermore, the generality of the synthesis method for MSU-Ge-2 will lead to a variety of elemental mesoporous semiconductors, such as Sn and Si and solid solutions thereof, where the composition and pore geometry can be controlled to enable the variation of their optoelectronic and mass transport properties.

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19. Materials and methods are available as supporting material on *Science* Online.
20. Upon heating the ion-exchanged material at low temperature (evacuated at 75°C), the ammonium counterions are largely removed from the mesopores according to elemental C, H, and N (*19*) and TG analyses. These results indicate that only 9.7% (w/w) of the surfactant remained inside the pores. Although the reason for this is not well understood, the result suggests that the remaining surfactant is anchored by strong interactions because a much higher temperature (~463°C) was required to remove it (Fig. 1B), leading to decomposition.
21. FT-IR spectroscopy of as-prepared MSU-Ge-2 shows bands at 1985 and 562 cm^{-1}, which is assigned to stretching mode and possible bending in-plane (rocking) mode of -GeH group, respectively (Fig. 1C, curve a). Exchange with NH_4^+ ions causes the stretching band of GeH to broaden and shift toward higher frequencies (2004 cm^{-1}) (Fig. 1C, curve b). The broadening of this absorption band is attributed to the different environments of Ge–H bonds (*27*). The FT-IR spectra of mesoporous MSU-Ge-2 lack the characteristic N-H stretching and bending band. Instead, bending corresponding bands of the -GeH_2 and -GeH groups appear at 2141, 2011, and 566 cm^{-1} (Fig. 1C, curve c).
22. The hexagonal unit cell parameter, a_H, was estimated according to the $a_H = 2/\sqrt{3}d_{100}$ equation, where d_{100} represents the d spacing of the (*100*) diffraction plane, equal to 4.16 and 3.97 nm for the as-prepared and NH_4^+-exchanged materials, respectively.
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24. The equivalent surface area of 1316 $m^2\ g^{-1}$ for the silicon dioxide (triatomic "SiO_2" formula) was obtained on the basis of molecular weight calculations and by assuming the equivalent triatomic "Ge_3" unit structure with surface area of 363 $m^2\ g^{-1}$.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/1130101/DC1
Materials and Methods
Figs. S1 to S4
References

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Time-Resolved Seismic Tomography Detects Magma Intrusions at Mount Etna

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The continuous volcanic and seismic activity at Mount Etna makes this volcano an important laboratory for seismological and geophysical studies. We used repeated three-dimensional tomography to detect variations in elastic parameters during different volcanic cycles, before and during the October 2002–January 2003 flank eruption. Well-defined anomalous low *P*- to *S*-wave velocity ratio volumes were revealed. Absent during the pre-eruptive period, the anomalies trace the intrusion of volatile-rich (≥4 weight percent) basaltic magma, most of which rose up only a few months before the onset of eruption. The observed time changes of velocity anomalies suggest that four-dimensional tomography provides a basis for more efficient volcano monitoring and short- and midterm eruption forecasting of explosive activity.

Repeated seismic tomography [four-dimensional (4D) tomography: in space and time] can be used to reveal variations of elastic properties within rock volumes. This approach has been used in geothermal and volcanic areas (*1*, *2*) and in hydrocarbon surveillance (*3*), in order to monitor gas concentration and migration and to track changes during exploitation, respectively. Seismic velocity variations depend on the characteristics of the rock (such as mineralogy, porosity, fracturing, and fluid saturation) and its physical conditions (such as temperature and pressure) (*4*–*7*). In a volcanic environment, the presence of fluids, cracks, and gas, or their temporal variations within the plumbing system, may also change the elastic properties drastically and suddenly (*8*–*10*).

Mount Etna, a basaltic stratovolcano located near a densely populated area, is commonly considered to exhibit only effusive eruptions. However, it is one of the few alkali-basaltic volcanoes for which several violent explosive (subplinian and plinian basaltic-type) eruptions are documented (*11*). Recently, from 27 October 2002 to 18 January 2003, a flank eruption (Fig. 1) was characterized by an intense explosive activity with column-forming fire fountains and fairly continuous tephra fallout. This peculiar activity was fed by the uprising of a new intrusion of volatile-rich basaltic magma, containing CO_2-rich gas of deep derivation (>10 km) (*12*, *13*). Because repeated seismic tomography may be a reliable indicator of the migration of fluids in active volcanoes (*2*), this approach may allow the detection of the transient presence of magmatic intrusions that are rich in gas and provide a valuable tool for future forecasting of Mount Etna's volcanic activity. However, some problems may arise when a tomographic study is applied to evaluate the temporal variations of elastic parameters, because the resolution of velocity models may differ by inverting contiguous short-period data sets. Moreover, the dimension of resolvable features may be higher than that of rapidly changing local anomalies, and normally we lack independent evidence for physical phenomena that change elastic parameters over time. Conversely, if a long-period data set is used in the tomographic analysis, the spatial distribution of the anomalies may be biased or obscured by averaged values when the volcano is characterized by highly eruptive dynamics.

In our study, the use of a large number of permanent seismic stations and the abundance of local earthquakes, occurring both before and during the eruptions, guarantee a consistent and high-resolution velocity model. We analyzed the seismicity (Fig. 2) recorded at the Mount Etna Istituto Nazionale di Geofisica e Vulcanologia–Sezione di Catania (INGV-CT) computerized permanent seismic network (Fig. 1) before and during the 2002–2003 flank eruption. The permanent network was composed of 45 stations, 5 of which were three-component short-period (1 s) stations and three of which were digital three-component broad-band (40 s) stations. On 6 November 2002, a temporary array composed of eight digital three-component broad-band (60 s) stations was integrated into the permanent network in the northern sector

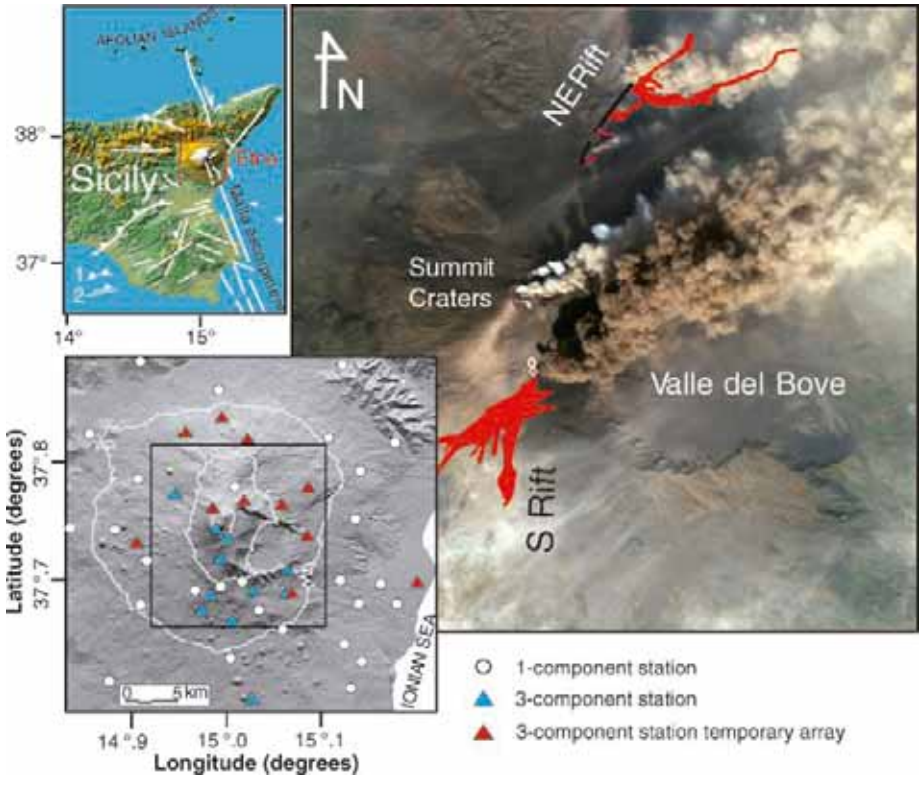

Fig. 1. (**Right**) Image of the summit of the Mount Etna volcano showing the ash plumes during one day of the 27 October 2002 to 18 January 2003 eruption [image acquired by Digital Globe's Quickbird-2 satellite (http://rst.gsfc.nasa.gov/Sect13/Sect13_4d.html)]. Eruptive fissures opened on the northeast and south rifts of the volcano. Black lines, eruptive fractures; red areas, lava flows. (**Top left**) Structural map of eastern Sicily showing the front of the Appenninic-Maghrebian chain (1) and the main faults (2). (**Bottom left**) Map of the Mount Etna area indicating the seismic stations operating during the study period. The black square indicates the summit of the volcano shown in the image on the right. The concentric white curves represent elevation contours at 1000-m intervals.

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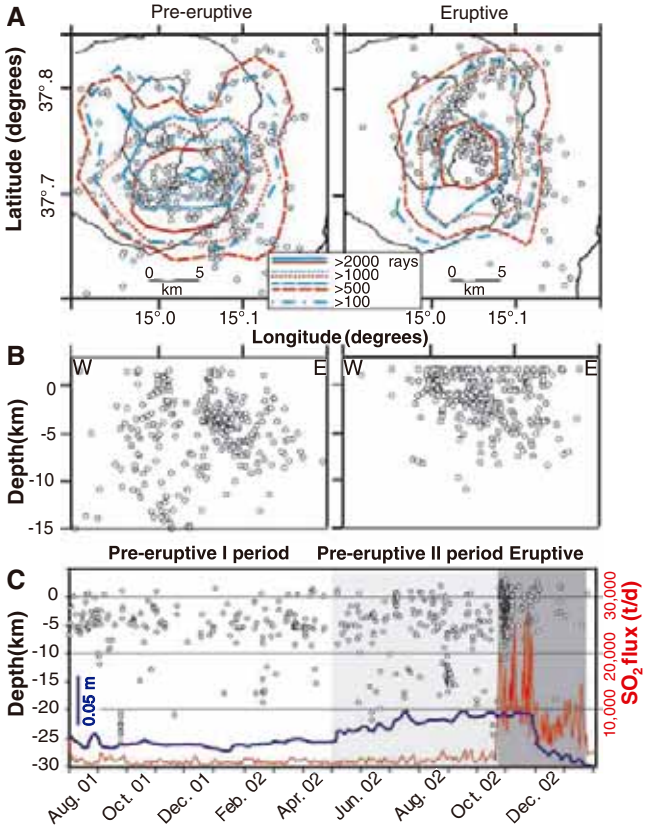
of the volcano. During the study period, some stations were moved, depending on the location of seismicity.

First, we performed a tomographic inversion of the whole data set (10 August 2001 to 18 January 2003) to define the 3D *P*-wave velocity (V_P) and the structure of the *P*- to *S*-wave velocity ratio (V_P/V_S). A total of 712 well-constrained earthquakes [root mean square (rms) time residuals ≤0.2 s; horizontal (Erh) and vertical (Erz) hypocentral location errors ≤1.0 km; azimuthal gap of the stations ≤120°], 8587 *P*-wave arrivals, and 2293 *S*-wave arrivals were inverted to model a grid 2 km by 2 km by 1 km with the use of SIMULPS-14 software (*14*). On the digital seismograms, the measured time pickings were precise to ~0.01 s, and phase arrivals were checked by polarization analysis, especially for the *S* waves. The starting velocity model was derived from the 3D model developed by Patanè *et al*. (*15*). The input value for the V_P/V_S ratio was 1.73, which is in agreement with the results of Laigle (*16*). After 10 iterations, the variance improvement was 34.7%, with a final rms value of 0.20 s (table S1). The reliability of the V_P and V_P/V_S models has been verified by the analyses of the full-resolution matrix (Fig. 3) and by synthetic tests [supporting online material (SOM) text]. This inversion allows the improvement of even the most recent tomographic results (*15*, *17*) and better definition of the shape and geometry of the upper portion of high-velocity V_P volume, interpreted as a main solidified intrusive body (*15*) (Fig. 3A). However, the most notable result concerns the detection of anomalous zones with low V_P/V_S values (*18*).

The V_P/V_S images, which present a satisfactory resolution [spread function (SF) ≤2] between the depths of –4 and 2 km (Fig. 3B), clearly highlighted two anomalous regions characterized by low V_P/V_S values (as small as 1.64). These were located in the central-southern and northeastern part of the volcanic edifice, where geodetic data modeled the dike intrusions of 2002–2003 (*19*, *20*) (Fig. 3B) and beneath the eruptive fracture systems (Fig. 1).

Because the V_P/V_S ratio can be very sensitive to factors that may change with time during the magmatic cycles, we separately analyzed the pre-eruptive (10 August 2001 to 25 October 2002) and the eruptive (26 October 2002 to 18 January 2003) seismicity. We further subdivided the pre-eruptive period into two time intervals because geochemical observations, ground deformation measurements obtained via Global Positioning System (GPS) technology, and seismicity evidence (Fig. 2C) (*20*) indicated that there was a recharging phase, which started in April



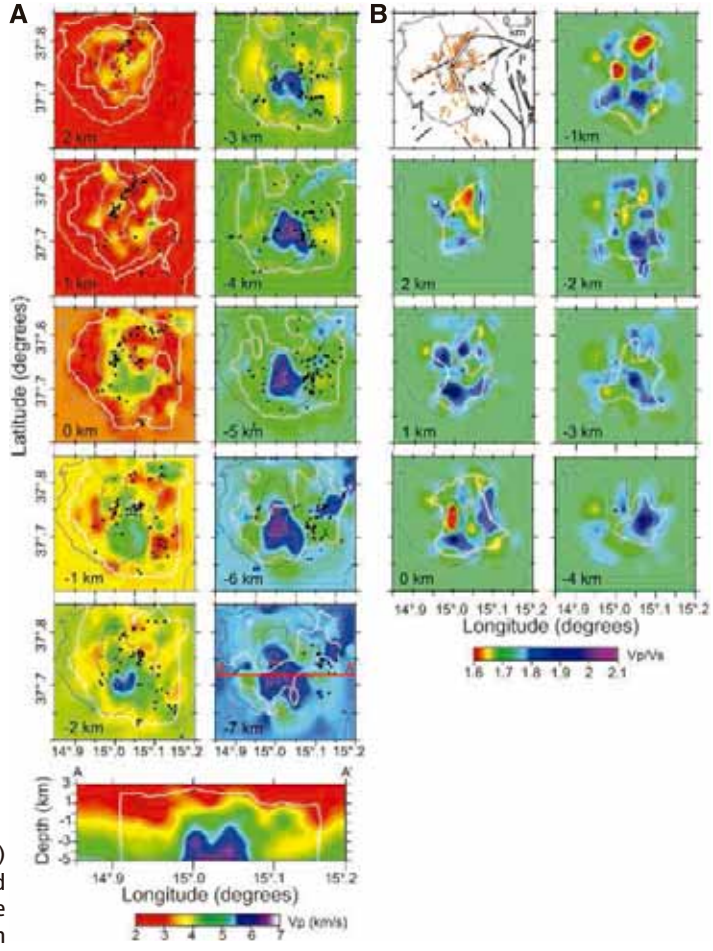


Fig. 2. (**Left**) Maps of (**A**) 1D epicentral locations (gray circles) and (**B**) related W-E cross sections across the summit craters for 712 selected earthquakes that were recorded during the pre-eruptive and eruptive periods. In (A), the sum of the rays contributing to the solution at each grid node that was defined for the inversion is represented by the density contours for both *P* (red lines) and *S* waves (blue lines). (**C**) The plot versus time of focal depths for the whole data set (gray circles), ground deformation time series related to a central-western GPS baseline (*20*) (blue line), and SO_2 flux daily measurements (red line). t/d, metric tons per day. Note (i) the increasing rate of ground deformation since April 2002 (light gray area); (ii) the slight increase in SO_2 flux since August; and (iii) the very high value of SO_2 flux (up to ~30,000 t/d) measured during the eruptive period (dark gray area). **Fig. 3.** (**Right**) Velocity models for V_P (**A**) and V_P/V_S (**B**) in the well-resolved layers. The white contours indicate the regions of the model with SF values <2 and where the resolution is good (SOM text) (*27*, *28*). The gray lines are elevation isolines (every 1000 m). The black dots in the Vp model (A) are the earthquakes relocated with the 3D velocity model. The W-E cross section of the V_P model crossing the grid center (red horizontal line) is reported in the –7-km layer. In the top left square of (B), historical eruptive fissures (orange lines), major faults (black lines), and the surface projection of the 2002–2003 dike intrusions (gray rectangles) modeled by geodetic data (*19*, *20*) are shown.

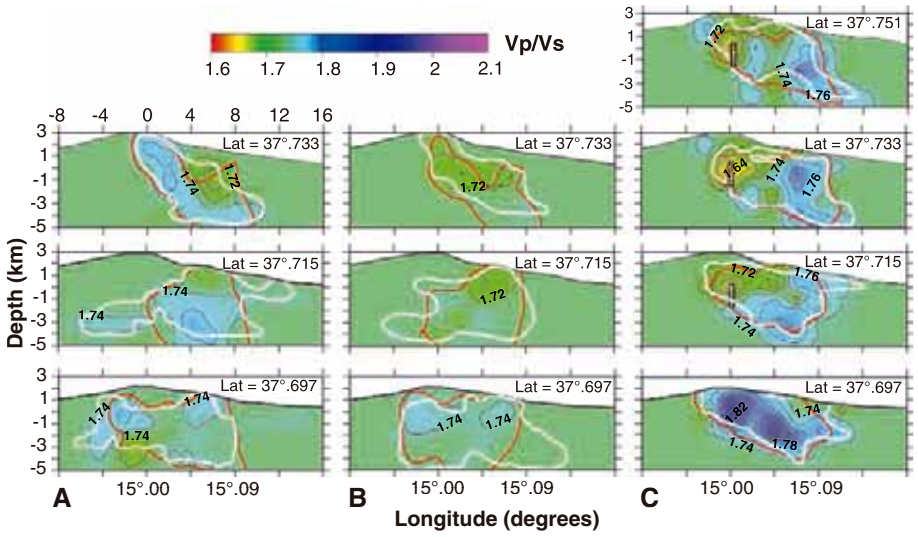


Fig. 4. W-E cross sections showing the variation of the V_P/V_S ratio during the pre-eruptive periods [(**A**) and (**B**)] and the 2002–2003 eruption (**C**). The vertical black rectangle in (C) indicates the location of the 2002–2003 dike intrusion, as modeled by geodetic data in the southern flank (*19*, *20*). The white and red lines indicate the well-resolved regions of the model with SF values <2 and derivative weight sum values >50, respectively (SOM text).

2002, following an approximate 8-month period of deflation at the end of the 2001 flank eruption.

For these inversions and for each epoch, we verified the reliability of the V_P/V_S models (SOM text) as described above, by using both the analyses of the full-resolution matrix (Fig. 4 and fig. S1) and synthetic tests (fig. S2). In west-to-east (W-E) cross sections for the different analyzed periods (Fig. 4), we observed a substantial change of V_P/V_S anomalies through time. During the 2002–2003 eruption, strong anomalies with low V_P/V_S values were revealed beneath the central-southern and northeastern parts of the volcanic edifice. Conversely, high or normal V_P/V_S values were found in the same region during the deflation after the end of the 2001 eruption (Fig. 4A) and the pre-eruptive recharging period (Fig. 4B). The time change of V_P/V_S anomalies revealed a variation of both fluid and fluid pressure in the shallow layers (depth <5 km), due to the uprising of magma from greater depth that started only a few months before the onset of the 2002–2003 eruption (*13*, *21*).

Geochemical and petrological features of the erupted lavas (*13*, *22*), and evidence for the expulsion of a large volume of pyroclastic products [40×10^6 m^3 to 50×10^6 m^3 with an explosive index of ~0.55 (*22*)] during the eruption, support the hypothesis that these low V_P/V_S volumes are related to the intrusion of volatile-rich [≥4 weight % (*12*, *13*)] basaltic magma.

During the eruptive period, we observed wide regions with high V_P/V_S anomalies mainly located beneath the eastern and southeastern flanks of the volcano (Fig. 4C). It is widely accepted that melt-filled inclusions result in a high V_P/V_S ratio (*8*–*10*), even if the effects of a partial melt on seismic wave velocities are still poorly known. However, the possibility of a partial melt cannot be entirely excluded from having occurred inside the regions of high V_P/V_S values, especially near the central craters and southward where the magma intruded during 2001 (*23*). We hypothesize that the high V_P/V_S anomalies are related to the rapid migration of fluids from the intrusion zone into the fractured regions beneath the eastern and southeastern flanks (*24*). In these regions, crack density increases because of the intense fracturing occurring both during the pre-eruptive and eruptive periods (see the location of seismicity in Fig. 2).

On the basis of the geophysical evidence presented here, we believe that the extensive application of 4D tomography could be widely used in volcanic monitoring and in the prediction of violent explosive eruptions generated by gas-rich magma. However, this technique requires high-quality, densely positioned, three-component seismic networks, which were not available during previous large eruptions at Mount Etna and are not in place at most volcanoes worldwide.

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18. For igneous rocks, laboratory experiments found that the V_P/V_S ratio generally decreases as temperature increases and increases as pressure increases. An increase in the V_P/V_S ratio is related to increases in temperature, fracture, and especially partial melt, whereas a decrease in the ratio can be associated with the presence of gas or supercritical fluids [see (*10*) and references therein].
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24. Theory predicts that the velocity decreases and V_P/V_S increases for increasing crack density (*4*). High V_P/V_S values affecting portions of fault zones, such as at the San Andreas Fault, are interpreted as evidence for the presence of overpressured fluids (*25*). The influence of pore pressure on seismic velocities is caused by the tendency of cracks to remain open when they are internally pressurized (*26*). Thus, fracturing and increasing pore pressure cause a relatively marked decrease of V_S but only a small decrease of V_P, which yields an increased V_P/V_S ratio. The concentration of fluids in a dike intrusion mainly occurs near its tip. Moreover, a rapid migration of fluids in the surrounding rock volume can also occur, especially when prefractured zones exist, leading to a high V_P/V_S ratio and to an increase of the seismicity (Figs. 2 and 4C).
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An Experimental Study of the Coloring Problem on Human Subject Networks

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Theoretical work suggests that structural properties of naturally occurring networks are important in shaping behavior and dynamics. However, the relationships between structure and behavior are difficult to establish through empirical studies, because the networks in such studies are typically fixed. We studied networks of human subjects attempting to solve the graph or network coloring problem, which models settings in which it is desirable to distinguish one's behavior from that of one's network neighbors. Networks generated by preferential attachment made solving the coloring problem more difficult than did networks based on cyclical structures, and "small worlds" networks were easier still. We also showed that providing more information can have opposite effects on performance, depending on network structure.

It is often thought that structural properties of naturally occurring networks are influential in shaping individual and collective behavior and dynamics. Examples include the popular notion that "hubs" or "connectors" are inordinately important in the routing of information in social and organizational networks (*1*, *2*). A long history of research has established the frequent empirical appearance of certain structural properties in networks from many domains, including sociology (*1*, *3*–*5*), biology (*6*, *7*), and technology (*8*). These properties include small diameter (the "six degrees of separation" phenomenon), local clustering of connectivity (*9*), and heavy-tailed distributions of connectivity (*10*). Theoretical models have sought to explain how some of these may interact with network dynamics (*11*).

The relationships between structure and behavior are difficult to establish in empirical field studies of existing networks. In such studies, the network structure is fixed and given, thus preventing the investigation of alternatives. A different approach is to conduct controlled laboratory studies in which network structure is deliberately varied.

We have been performing human subject experiments in distributed problem-solving from local information on a variety of simple and complex networks. Subjects each simultaneously control a single vertex in a network of 38 vertices and attempt to solve the challenging graph coloring problem (*12*) on the network. In this problem, the collective goal is for every player to select a color for their vertex that differs from the colors of all of their network neighbors. The number of colors made available is the minimum necessary to color the entire network without conflicts (edges connecting two vertices with the same color), known as the chromatic number of the network.

The graph coloring problem is a natural abstraction of many human and organizational problems in which it is desirable or necessary to distinguish one's behavior from that of neighboring parties. As a specific scenario, consider the problem faced by faculty members scheduling departmental events—recurring classes, one-time seminars, exams, and so on—in a limited number of available rooms. We can view the events to be scheduled as the vertices in a network, with an edge connecting any pair of events that temporally overlap, even partially. Clearly, two such events must be assigned to different rooms or "colors," thus yielding a natural graph coloring problem. Furthermore, even when there is a centralized first-come, first-serve sign-up sheet for rooms, this mechanism is simply the starting point for the negotiation of a solution, and the problem is still solved in a largely distributed fashion by the participants: Faculty members routinely query the current holder of a room whether they might be able to switch to a different room, whether their event will really require their entire time slot, and the like. Other coloring-like problems arise in a variety of social activities (such as selecting a cell phone ringtone that differs from those of family members, friends, and colleagues); technological coordination [selecting a channel unused by nearby parties in a wireless communication network (*13*, *14*)]; and individual differentiation within an organization (developing an expertise not duplicated by others nearby). Graph coloring also generalizes many traditional problems in logistics and operations research (*12*).

The coloring problem was chosen for both its simplicity of description and its contrast to other distributed network optimization problems. Unlike the well-studied studied navigation or shortest-paths problem, optimal coloring is notoriously intractable from the viewpoint of even centralized computation (*12*, *15*). In fact, even weak approximations (in which many more colors than the chromatic number are permitted) are known to be equally difficult (*16*, *17*).

We report here on the findings from two extensive experimental sessions held in January 2006 with 55 University of Pennsylvania undergraduate students (*18*). Subjects were given a series of coloring experiments in which the network had one of six topologies, each chosen according to recently proposed models of network formation (Fig. 1 and Table 1). Three of these six begin with a simple cycle and then add a varying number of randomly chosen chords while preserving a chromatic number of two. These "small worlds" networks (*9*, *19*) are intended to model the mixture of local connectivity (as induced by geography) with long-distance connectivity (as induced by travel or chance meetings) often found in social and other networks. The fourth cycle-based network adopted a more engineered or hierarchical structure, with two distinguished individuals having inordinately high connectivity. The fifth and sixth networks were generated according to the well-studied preferential attachment

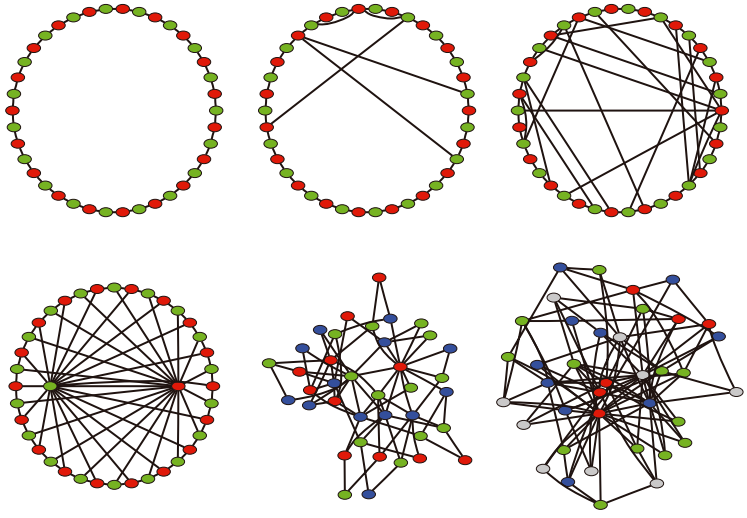

Fig. 1. Network topologies with sample colorings found by subjects. From left to right and top to bottom: simple cycle, 5-chord cycle, 20-chord cycle, leader cycle, and preferential attachment with two and three links initially added to each new vertex.

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model (*10*), in which vertices already highly connected are more likely to receive further connections as the network is formed incrementally. Such networks are known to generate a number of structural properties frequently documented empirically, including the presence of highly connected "hubs."

Six or seven trials (*20*) of each of the six networks were performed under varying informational conditions. Subjects sat at workstations running a browser-based client of a distributed computer system built for the experiments. The interface provided each subject with a local view (including their own color and those of their neighbors) of the current state (Fig. 2). In a minority of trials, subjects were given a global view. Subjects were familiarized with the coloring problem but given no guidance on how to play; subjects could update their colors at any time from a fixed menu that provided the minimum number of colors required to solve the problem for the given network. The experimental protocol forbade all communication outside the confines of the system, and physical partitions prevented subjects from seeing beyond the information view provided on their own workstation. In accordance with standard practices in behavioral game theory and economics (*21*), participants received $5 for each experiment in which they were "successful" (*22*).

Subjects could indeed solve the coloring problem across a wide range of network structures. Of the 38 experiments we conducted, 31 (82%) resulted in an optimal coloring of the network in less than the allotted 5 min (300 s), with the mean completion time of the solved networks only 82 s (SD, 75 s) and the median just 44 s, indicating considerable skew toward low solution times. All six of the network structures were solved at least twice when subjects could see only their neighbors' color choices (low-information view).

Collective performance was strongly affected by network structure. The networks generated by preferential attachment proved considerably more difficult than any of the cycle-based networks: Six of the seven experiments that ended without an optimal coloring after 5 min were on networks of the former type, and the mean experiment duration [which includes 300-s values for unsolved networks (*23*)] for preferential attachment graphs was higher than for all others (Table 1). The duration times for the cycle-based networks and those for the preferential attachment networks passed a two-tailed, unequal variance t test for different means at $P = 0.03$. Within the cycle-based family, there was a monotonic relationship between solution time and network average distance (the average shortest distance, measured in number of links traveled, across all pairs of vertices), with smaller average distance leading to shorter solution times. (For the cycle-based networks, the correlation between aver-

Table 1. For each of the six experimental networks, the first six columns provide statistics summarizing structural properties, including the chromatic number (smallest number of colors required for solution), and statistics on the distribution of the degree (number of links) of each vertex. Network average distance is the average shortest-path distance, measured in number of links traveled, over all pairs of vertices. Also displayed are the average experiment duration for each network, along with the fraction of trials on which it was solved within 300 s and the number of steps (measured in color changes) for a natural distributed computer heuristic. Pref. att., preferential attachment.

	Graph statistics								
	Colors required (No.)	Min. links (No.)	Max. links (No.)	Avg. links (No.)	SD	Avg. distance (No. of links)	Avg. experiment duration (s) and fraction solved		Distributed heuristic (No. of color changes)
Simple cycle	2	2	2	2	0	9.76	144.17	5/6	378
5-chord cycle	2	2	4	2.26	0.60	5.63	121.14	7/7	687
20-chord cycle	2	2	7	3.05	1.01	3.34	65.67	6/6	8265
Leader cycle	2	3	19	3.84	3.62	2.31	40.86	7/7	8797
Pref. att., $\nu = 2$	3	2	13	3.84	2.44	2.63	219.67	2/6	1744
Pref. att., $\nu = 3$	4	3	22	5.68	4.22	2.08	154.83	4/6	4703

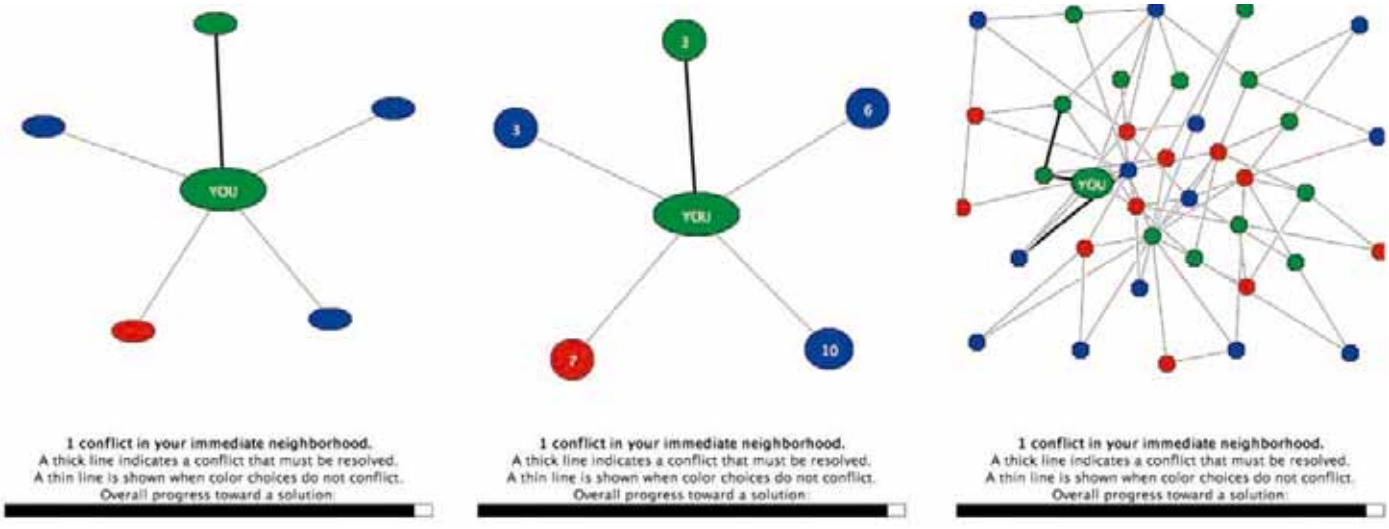

Fig. 2. In the low-information view (**left**), subjects could see only the color they chose for their own vertices and the colors of their immediate neighbors in the graph. The medium-information view (**center**) is similar, but each neighbor is labeled by its number of links. In the high-information view (**right**), each subject could see the current color choices of the entire network. The text at the bottom of each screenshot reads as follows: "1 conflict in your immediate neighborhood. A thick line indicates a conflict that must be resolved. A thin line is shown when color choices do not conflict." The bar at the bottom of each screen gave subjects an indication of global progress toward a solution.

age distance and experiment duration was 0.45, with $P = 0.02$.) Thus, the highly symmetric leader cycle (average distance 2.3) led to the fastest optimal colorings, the simple cycle (average distance 9.8) to the slowest. The addition of random chords to the simple cycle systematically reduced solution time. Although the addition of chords makes the problem more difficult from the isolated viewpoint of any individual subject (because they must now coordinate with a potentially larger number of neighbors but still are permitted only two colors), it apparently makes the collective problem easier by reducing the number of links coloring conflicts must travel to be resolved. This establishes a second and rather different problem [along with navigation (*11*, *24*)], for which reduced average distance seems to have a beneficial influence on collective behavior.

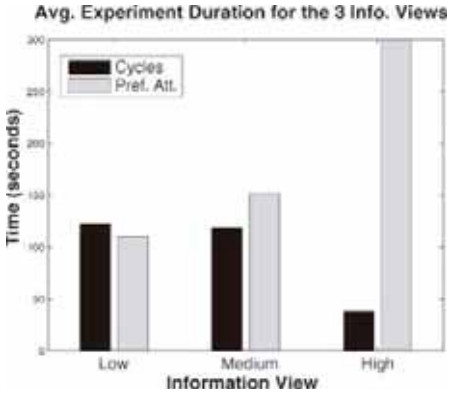

Fig. 3. More information resulted in a decrease in experiment duration for the four cycle-based graphs and an increase in duration for the two preferential attachment graphs. If we introduce an ordinal variable assuming a value of 1 for the low-information condition, 2 for the medium-information condition, and 3 for the high-information condition, the correlation between this variable and experiment duration is –0.40 for the cycle-based networks ($P = 0.04$), and 0.65 ($P = 0.02$) for the preferential attachment networks.

It is interesting to compare the influence of network structure on collective human behavior with its influence on natural distributed heuristics. Here, we consider the simplest such heuristic, in which a vertex v is randomly selected among those with a color conflict (that is, a neighboring vertex of the same color). If there are one or more unused colors in the neighborhood of v, one is selected at random for v, thus eliminating the conflict; if not (conflict is inevitable), a new color for v is simply chosen randomly among all possible colors. Because the direct comparison of experimental duration for human populations with computation time is not meaningful, in the rightmost column of Table 1 we report the mean number of color changes (averaged over 10,000 trials) required for this heuristic to successfully color each of the six networks. (We note that for the human subjects, the number of color changes made by the population and experiment duration are proportional and nearly perfectly correlated.) The differences with the collective human behavior are striking, with the order of difficulty within the cycle family exactly reversed (lower average distance increases difficulty for the heuristic), and the preferential attachment networks being relatively easy for the heuristic.

Our final result concerns the effects of varying the locality of information provided to subjects. Our system provided subjects one of three information views during play. Two of these were highly localized, allowing participants to see only their own and neighboring colors, with one of them additionally providing static connectivity information about neighbors (Fig. 2). A minority of experiments used a third information view that allowed subjects to see the global coloring state at all times. In each experiment, the same type of information view was given to all subjects. The most striking finding again highlights apparently strong differences between our two main categories of networks: Although increasing the amount of information provided sharply reduced solution times for cycle-based networks, it sharply increased it for preferential attachment (Fig. 3). For the cycle-based networks, there are only two possible proper colorings (each a cyclic shift of the other), and subjects seem to have a strong understanding of the collective effort required to rapidly coordinate and converge to one of them when provided with a global view (Fig. 4). In contrast, no such common understanding appears to exist for preferential attachment, and the global information view seems to greatly hamper subjects. All four trials of preferential attachment graphs with global views ended without solution after 5 min. Possible explanations include "information overload" due to the apparent complexity of the networks or their visual layout, combined with the rapid dynamics of the global color selection process. Alternately, it could simply be that time spent by subjects examining the activity in more distant regions of the network distracts them from attending to their own local subtask in the global coordination problem, thus slowing collective solution. With further study, such findings may have implications for areas such as information sharing across large organizations and the design of user interfaces for complex systems for multiparty coordination.

The discussion so far has emphasized collective behavior and performance, but it is also of interest to understand the individual strategies for play used by subjects. Toward this goal, we can apply both the detailed experimental data (which logs every color change by every player, along with their times of occurrence, for each experiment) and the self-reports of the subjects themselves, who were given an exit survey asking them what strategies they employed. These surveys reveal frequent and independent adoption

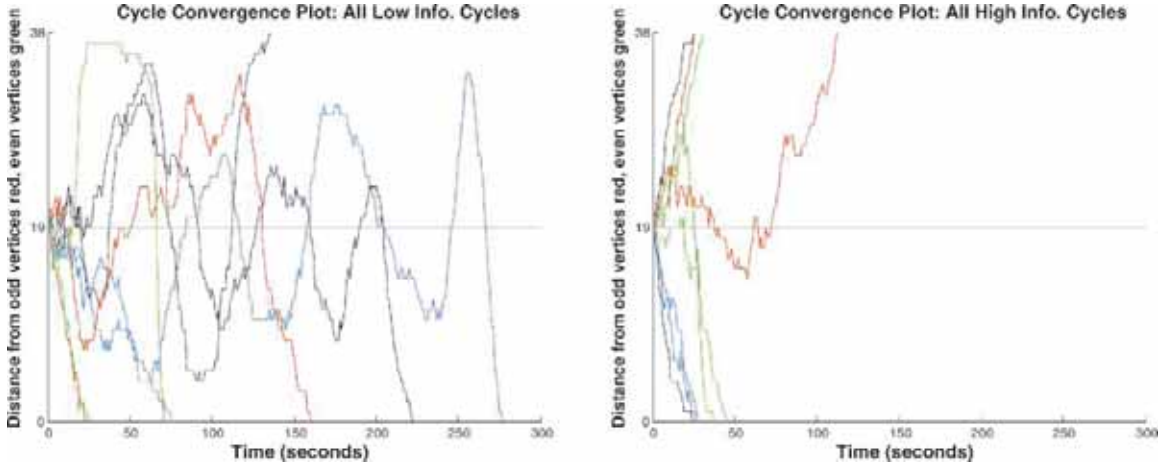

Fig. 4. Population convergence to one of the two possible proper colorings for the four cycle-based graphs. The *y* axis measures distance from the two colorings (in terms of number of disagreements), and the *x* axis measures time. Points below the horizontal line at $y = 19$ are closer to one of the two solutions, whereas points above this line are closer to the other. A *y* axis value of 0 or 38 indicates a completed experiment in which the corresponding coloring was found by the population. All experiments begin equidistant ($y = 19$) from both solutions. In the experiments under the low-information view (**left**), the population often oscillates between approaches to the two solutions, whereas in the high-information view experiments (**right**), there is rapid convergence to one of the two solutions with almost no oscillation.

of certain natural heuristics. These include choosing colors that will result in the fewest local conflicts (mentioned on 11 surveys), as well as attempting to avoid conflicts with neighbors with high connectivity (mentioned on 39 surveys, and obviously applicable to only those two information views that revealed such neighboring information), presumably on the logic that highly connected vertices present the most constrained and difficult problems for subjects. Surveys and the experimental data also revealed a number of instances of signaling behavior by subjects, but here there was less consistency. Some subjects clearly alternated between two colors that were unused in their neighborhood in an attempt to inform neighbors of this fact. Others would alternate between colors in an attempt to call attention to conflicts. Although such signaling behaviors are apparent in the data, it is unclear whether they ever had their intended effects. Many subjects also reported introducing conflicts into their local neighborhood even when they had an available color, in an attempt to perturb the global state from a perceived stasis or local minimum. Even excluding perturbations introduced 2 s or less after the absence of any local conflicts (to account for reaction time), the 38 experiments together had 181 such incidents. This behavior might be viewed as a human analog of the deliberate injection of randomization or "thermal noise" into common optimization algorithms such as simulated annealing. Further work is needed to integrate these observations with statistical methods applied to the experimental data, in order to develop plausible stochastic models of individual behavior in the coloring problem. Ideally such models, when run in multiple independent copies, could predict which networks would be easy or difficult for human populations.

Although the results presented here are suggestive, they are limited in a variety of important ways. The human subject networks were small, a perhaps necessary consequence of the carefully controlled, simultaneous play experiments. It is tempting to contemplate Web-based studies (*25*) on a much larger scale, which will require addressing incentives, attrition, communication, and many other issues. The network topologies examined here were but a sampling of the rich space of possibilities and recent network formation models. Rather than imposing a chosen network structure on subjects, it would also be interesting to consider scenarios in which the subjects themselves participated in the network formation process, while still allowing some variability of structure. Future work should consider an even wider range of natural collective problems and activities. Candidates include problems of agreement or consensus rather than differentiation, and problems involving the formation of local teams or subgroups specifying certain properties (such as being fully connected or having at least one member of each of a fixed number of types or roles).

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20. For each network structure, trials were conducted under three information conditions. For each network, we also examined two different incentive or payment schemes for subjects. The variation across these two design variables yielded $3 \times 2 = 6$ trials of each network. Two networks were given an additional trial to examine potential learning effects, yielding a total of seven trials. Details in (*26*).
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26. Materials and methods are available as supporting material on *Science* Online.

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Insignificant Change in Antarctic Snowfall Since the International Geophysical Year

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Antarctic snowfall exhibits substantial variability over a range of time scales, with consequent impacts on global sea level and the mass balance of the ice sheets. To assess how snowfall has affected the thickness of the ice sheets in Antarctica and to provide an extended perspective, we derived a 50-year time series of snowfall accumulation over the continent by combining model simulations and observations primarily from ice cores. There has been no statistically significant change in snowfall since the 1950s, indicating that Antarctic precipitation is not mitigating global sea level rise as expected, despite recent winter warming of the overlying atmosphere.

Global sea level (GSL) has been increasing by 1.7 mm year^{-1} over the past century (*1*) and 2.8 mm year^{-1} over the past decade (*2*). One of the greatest uncertainties in predictions of GSL rise is the contribution of the Antarctic ice sheets (*3*). The Antarctic ice budget is balanced by the buildup of snowfall in the interior and wastage due to

melting and calving of ice along the coastal margins. Future scenarios from global climate models (GCMs) suggest that Antarctic snowfall should increase in a warming climate, mainly due to the greater moisture-holding capacity of warmer air (*4*), partially offsetting enhanced loss at the ice sheet peripheries. Perplexing temperature trends have been reported over Antarctica since continuous monitoring began with the International Geophysical Year (IGY) in 1957–1958, varying by the season, the region, and the time period analyzed (*5*, *6*). A recent study suggests a strong tropospheric warming signal has been manifested over Antarctica during winters since the early 1970s (*7*), the season during which much of the continent receives its maximum snowfall (*8*). Satellite-based ice velocity and altimetry measurements indicate that the West Antarctic Ice Sheet (WAIS) has been thinning over the past decade, with a contribution to GSL rise of 0.13 to 0.16 mm year^{-1} (*9*, *10*), consistent with widespread melting of ice sheet grounding lines (*11*). In light of these studies, it is essential to assess whether Antarctic snowfall has been increasing.

The latest studies using global and regional atmospheric models to evaluate changes in Antarctic snowfall indicate that no statistically significant increase has occurred since ~1980 over the entire grounded ice sheet, WAIS, or the East Antarctic Ice Sheet (EAIS) (*12*–*14*). A validation of the modeled-versus-observed changes (*12*) suggested that the recent model records are more reliable than the earlier global model records that inferred an upward trend in Antarctic snowfall since 1979 (*15*). The new studies also showed that interannual snowfall variability is considerable; yearly snowfall fluctuations of ±20 mm year^{-1} water equivalent (WEQ), i.e., ±0.69 mm year^{-1} GSL equivalent, are common (*12*) and might easily mask underlying trends over the short record. It is necessary to extend the snowfall record back to the IGY so that (i) trends can be assessed within a longer context, (ii) the snowfall record can be compared with the entire instrumental temperature record over Antarctica, and (iii) a 50-year benchmark for GCM evaluation is available.

The small volume of meteorological data over the Southern Ocean and Antarctica renders modeled snowfall amounts highly questionable before the modern satellite era (~1979) (*13*, *16*). The only other records of snowfall variability before 1979 are from ice cores, snow pits, and precipitation gauges. The spatial coverage of these data has been too sparse to accurately assess snowfall accumulation over the entire continent. However, in recent years scores of new ice core records have become available, due in large part to the International Transantarctic Scientific Expedition (ITASE), a multinational field program aimed at reconstructing the recent climate history of Antarctica through ice coring and related observations along an extensive network of traverses (*17*). In this study, we used these new records together with existing ice cores, snow pit and snow stake data, meteorological observations, and validated model fields to reconstruct Antarctic snowfall accumulation over the past 5 decades.

Each observational record is representative of an area surrounding it (a "zone"), the size of which depends on the atmospheric circulation, the interaction of wind with topography, and the time scale considered. Our method used meteorological model reanalysis fields to determine zones of snowfall coherence that correlate with the individual records at annual time scales. Assuming these zones adequately cover most of the continent given the available observational records, this information can be used to synthesize the observations into a continent-wide record of snowfall accumulation in a self-consistent manner. The model reanalysis data set we used is the European Centre for Medium-Range Weather Forecasts 40-Year Reanalysis (ERA-40) (*18*). We defined snowfall accumulation from ERA-40 precipitation fields that were adjusted to match long-term observed accumulation records (*19*). Precipitation dominates snowfall accumulation variability over Antarctica at model grid scales (*8*, *15*). ERA-40 precipitation was compared to independent observed accumulation records for overlap periods and shown to largely reproduce the interannual snowfall accumulation variability and

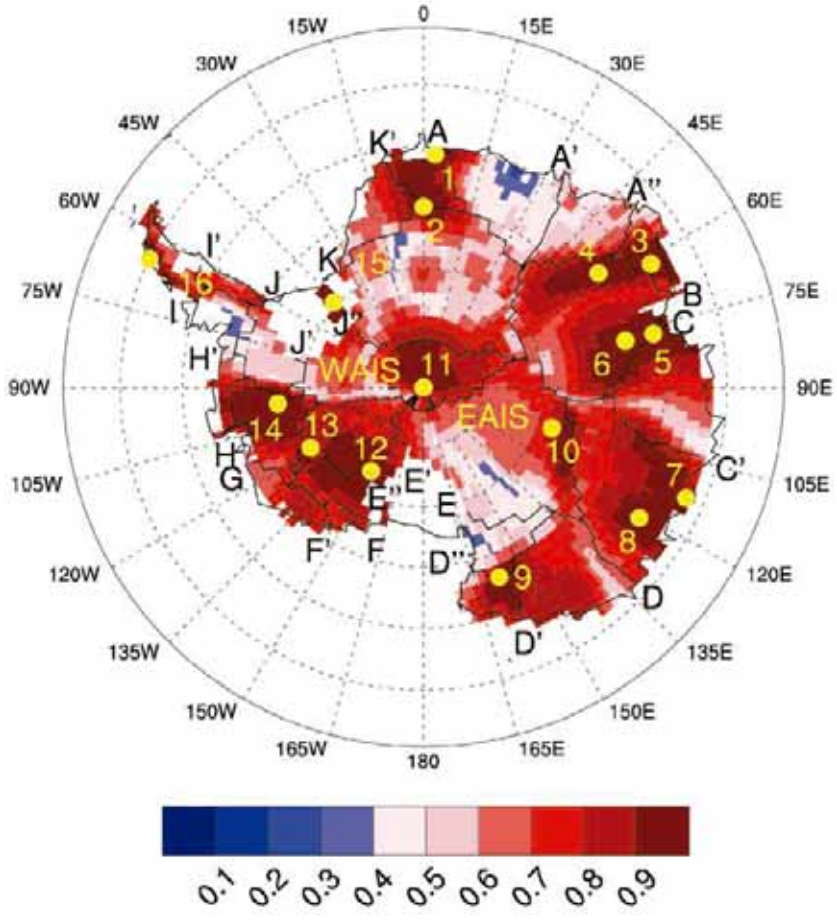

Fig. 1. The composite map of the maximum absolute value of the Pearson's correlation coefficient ($|r|$) resulting from correlating the ERA-40 1985–2004 percentage annual snowfall accumulation change (with respect to the 1985–1994 mean) for the grid box containing each of the 16 observation sites (yellow dots and numbers) with every other 1°-by-1° grid box over Antarctica (i.e., this map is a composite of 16 maps). Pink and red colors have correlations at $P < 0.01$. The black lines delineate ice drainage basins (*20*), which are identified alphabetically by the black letters where they intersect the grounding line. Detailed information about the observation sites is included in (*19*).

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trends, justifying its use for this study (*19*). Figure 1 shows a composite map of the maximum correlation coefficient obtained by correlating the ERA-40 simulated percentage annual precipitation anomaly at the grid point closest to each core with every other grid point. Correlations greater than 0.5 ($P < 0.01$) occur over most of the grounded ice sheet, indicating that the zones of spatial coherence from the available observational records cover nearly the entire continent. We used this robust relationship to synthesize the observational data into a series of continent-wide snowfall accumulation maps for the period before 1985, when the precipitation variability simulated by ERA-40 is questionable (*12*). The result is a 5-decade time series of snowfall accumulation over the grounded ice sheet; the first 3 decades are inferred from observational records, and the final 2 decades from ERA-40. A detailed description of the methodology is given in (*19*).

The spatial distribution of the 50-year average annual snowfall accumulation (Fig. 2A) closely resembles the glaciological estimate of Vaughan *et al*. (*20*). The mean for the grounded ice sheet is 182 mm year^{-1} WEQ, larger than the value of 149 mm year^{-1} WEQ from the Vaughan map. A subsequent analysis (*21*) suggested that the Vaughan map underestimated coastal accumulation and that a more realistic estimate is 171 mm year^{-1} WEQ. Overall, our mean annual snowfall accumulation is at the high end of published estimates [119 to 197 mm year^{-1} WEQ (*13*, *22*)] but may be realistic in light of recent findings.

The percentage differences of annual snowfall accumulation for each decade with respect to the 50-year mean (Fig. 2A) are shown in Fig. 2, B to F. There are regions of both positive and negative change in all 5 decades, but no continental-scale changes of either sign dominate any period. The amplitude of the changes in Fig. 2, B to D, the decades reconstructed from ice cores, is slightly dampened compared with the final two decades (Fig. 2, E and F). This is partly due the reconstructed data having smaller interannual variability than the model data; however, this does not affect the sign of the changes and has little impact on the results at basin and continental scales (*19*). There is no widespread signal of increased snowfall accumulation over the EAIS for 1995–2004 that would suggest a contribution to the recently reported thickening (*23*). The 1995–2004 changes are mostly negative over WAIS, where net ice sheet thinning is occurring (*9*, *10*). The statistical uncertainty associated with the change at each grid point (due to the decadal variability and methodology) is typically about 4 to 8%, enough to overwhelm the decadal changes in most places.

The time series of snowfall accumulation inferred from Fig. 2, B to F, and averaged over EAIS, WAIS, and the entire grounded ice sheet is shown in Fig. 3. All three regions are characterized by a steady upward trend from the beginning of the record through the early 1990s and then a downward trend thereafter that is most marked over WAIS (22 mm year^{-1} WEQ for the past decade compared with the prior decade). However, this change has low statistical significance ($P = 0.16$), indicating that decadal fluctuations of this magnitude (~7% of the 50-year mean) are probably common over WAIS. The upward trend over the ice sheets

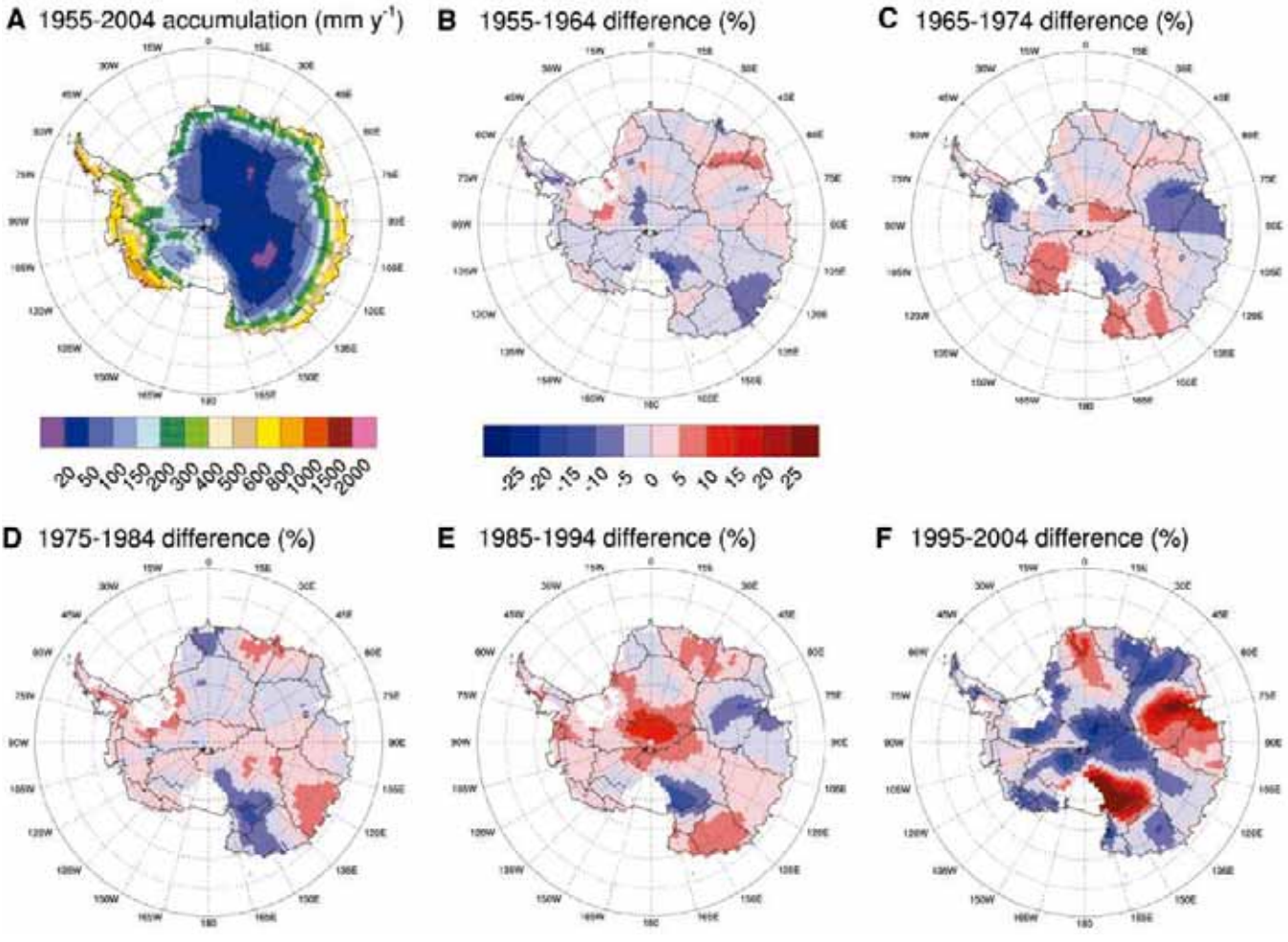

Fig. 2. (**A**) 50-year mean annual snowfall accumulation (mm year^{-1} WEQ). (**B** to **F**) Differences between mean annual snowfall accumulation for decade indicated and 50-year mean, expressed as a percentage of the 50-year mean. The scale shown in (B) applies to (B) to (F). The mean accumulation, trends, and uncertainty are quantified for each basin in (*19*).

before the most recent decade corroborates earlier studies that used regional records (*24*, *25*). Over EAIS, WAIS, and the grounded ice sheet, there are no statistically significant trends in snowfall accumulation over the past 5 decades, including recent years for which global mean temperatures have been warmest (*26*). We performed several experiments to test the sensitivity of the results in Fig. 3 by adjusting parameters within our methodology and by using other methods to reconstruct the accumulation, and the results were very robust (*19*).

Our findings are somewhat inconsistent with Davis *et al*. (*23*), who inferred from satellite altimetry data that an increase in snowfall accumulation was the primary cause of net thickening over EAIS for 1992–2003. One reason for the discrepancy may be that their radar data did not extend southward of 81.6°S, a region with strong downward trends in the past decade (Fig. 2F). Another factor may be their methodology. Zwally *et al*. (*10*) found a thickening over EAIS from satellite altimetry for a similar period that was a factor of 3 smaller than the value from the Davis study, arguing that their method more accurately accounts for firn compaction and interannual variability of the surface height. Lastly, because snowfall typically adjusts to climate change on much shorter time scales than the underlying glacial ice (*27*), a linear thickening trend as reported in the Davis study could be interpreted to mean that snowfall accumulation from 1992–2003 was stepwise higher than at some time in the past, when the accumulation rate and the ice sheet dynamical response were in equilibrium. In that case, the results of Davis *et al*. (*23*) may actually suggest that snowfall accumulation over EAIS has changed little in the past decade, consistent with our assessment. Despite our disagreement as to the causality, we do not dispute that altimetry indicates a clear thickening signal over EAIS (*10*, *23*) that mitigates sea level rise.

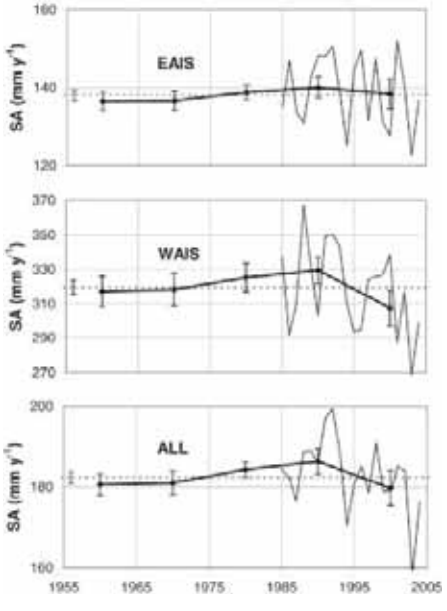

Fig. 3. Time series of decadal mean of annual snowfall accumulation (mm year^{-1} WEQ) for 1955–2004 for EAIS, WAIS, and the grounded ice sheet (ALL), calculated as described in the text. The annual accumulation is also shown for the past 2 decades, the period for which ERA-40 is used. The dotted line represents the 50-year mean. The basins that define EAIS, WAIS, and ALL are given in (*19*). Uncertainty bars are $\pm 1\sigma$ per our methodology (*19*). The uncertainty bar at the far left of each graph is for the 50-year mean.

The implications of our findings are categorized into two general ideas.

1) Interannual and interdecadal snowfall variability must be more seriously considered when assessing the rapid ice volume changes that are occurring over Antarctica. With regard to interannual variability, consider a recent estimate of Antarctic ice sheet mass loss that is the equivalent of 0.4 ± 0.2 mm year^{-1} GSL rise for 3 years (2002–2005) from satellite-derived time-variable gravity measurements (*28*). Antarctic-wide annual snowfall accumulation decreased by about 25 mm y^{-1} WEQ, or about 0.86 mm year^{-1} GSL rise, between calendar year 2002 and 2003 (Fig. 3), suggesting that the gravity fluctuations could be heavily influenced by interannual snowfall variations.

With regard to interdecadal variability, the ERA-40 snowfall accumulation is about 22 mm year^{-1} WEQ lower over WAIS for the past decade (1995–2004) compared with the previous decade (1985–1994) (Fig. 3), the GSL equivalent of 0.18 mm year^{-1}. This signal is of the same order as the 47 Gton (0.13 mm year^{-1} GSL equivalent) mass imbalance reported for WAIS (defined by a slightly different area) from satellite radar altimetry for roughly the past decade (*10*). In neither decade is the snowfall accumulation statistically significantly different from the 50-year WAIS mean, suggesting that such fluctuations are normal. The cause of the recent mass imbalance will remain unclear until a longer satellite record is available, but it may be partly related to accumulation variability.

2) Antarctic snowfall is not currently compensating for the oceanic-induced melting at the ice sheet periphery. If anything, our 50-year perspective suggests that Antarctic snowfall has slightly decreased over the past decade, while global mean temperatures have been warmer than at any time during the modern instrumental record (*26*). Radiosonde and ERA-40 temperature data indicate a uniform winter warming trend in the mid-troposphere over Antarctica since the early 1970s, but seasonally averaged ERA-40 precipitation data suggest that there has been no commensurate increase in winter snowfall since at least 1985 (*12*). These findings suggest that atmospheric circulation variability, rather than thermodynamic moisture increases, may dominate recent Antarctic snowfall variability.

Our technique of synthesizing observational records with model reanalysis has provided a coherent record of Antarctic-wide snowfall accumulation variability extending back before the modern satellite era. As more and improved (e.g., ground-penetrating radar) accumulation records become available, it will be possible to revisit this study with greater accuracy. A longer (1 to 2 centuries) reconstruction was not possible because of the limitations of the current data set but clearly is necessary to better understand the multidecadal Antarctic accumulation variability. Satellite-based techniques show great promise for precisely measuring Antarctic ice mass changes. It is critical to extend these records to distinguish thickening or thinning signals from snowfall variability.

Our results indicate that there is not a statistically significant global warming signal of increasing precipitation over Antarctica since the IGY, inferring that GSL rise has not been mitigated by recently increased Antarctic snowfall as expected. It may be necessary to revisit GCM assessments that show increased precipitation over Antarctica by the end of this century in conjunction with projected warming (*29*). Vigorous efforts are needed to better understand this remote but important part of the planet and its role in global climate and sea level rise.

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Supporting Online Material

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Materials and Methods
References

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Divergent Induced Responses to an Invasive Predator in Marine Mussel Populations

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Invasive species may precipitate evolutionary change in invaded communities. In southern New England (USA) the invasive Asian shore crab, *Hemigrapsus sanguineus*, preys on mussels (*Mytlius edulis*), but the crab has not yet invaded northern New England. We show that southern New England mussels express inducible shell thickening when exposed to waterborne cues from *Hemigrapsus*, whereas naïve northern mussel populations do not respond. Yet, both populations thicken their shells in response to a long-established crab, *Carcinus maenas*. Our findings are consistent with the rapid evolution of an inducible morphological response to *Hemigrapsus* within 15 years of its introduction.

Anthropogenic introductions increasingly bring organisms into contact that have no shared evolutionary history, which results in novel interactions between non-native and native competitors, prey, and predators (*1*). These novel species combinations create potentially strong selection pressure that can drive evolutionary change of heritable traits (*1*–*3*). Although several studies have shown that invaders can evolve rapidly in a novel, invaded environment (*1*), examples of invader-driven rapid evolutionary change in native species are rarer (*1*, *3*, *4*). Rapid evolutionary change may particularly influence the ability of native prey to recognize and respond to novel invasive predators with inducible morphological defenses.

Inducible defenses are the expression of alternative forms (phenotypic plasticity) by organisms in response to cues from a predator or competitor. Some commonly noted inducible defenses include shape changes in barnacles, spines on bryozoans and cladocerans, thickened shells of mollusks, defensive chemicals in plants, and morphological and behavioral characters in anuran tadpoles (*5*, *6*). Although selection may act on inducible defenses (*5*), in terms of both the degree of plasticity (*7*) and the prey's capacity to recognize cues from predators (*8*, *9*), to date there have been no examples of an invasive species driving the rapid evolution and emergence of an inducible morphological response. To test for the evolution of predator recognition and expression of inducible morphological defenses in a marine mussel (*Mytilus edulis*), we juxtaposed the induced defenses of two mussel populations having different historical contact with two invasive crab predators.

The Asian shore crab, *Hemigrapsus sanguineus*, was first reported in North America in New Jersey in 1988 and currently ranges from North Carolina to the midcoast of Maine, U.S.A. (*10*, *11*). *M. edulis* is a large component of *H. sanguineus*' diet (*12*), but perhaps because this is a novel predator in the North Atlantic Ocean, nothing is known about inducible defenses in mussels to this crab. A longer term resident of New England, the green crab, *Carcinus maenas*, was introduced from Europe to the Mid-Atlantic United States in 1817 and currently ranges from New Jersey, U.S.A., to Prince Edward Island, Canada (*13*). *C. maenas* has had substantial impacts on native communities throughout its introduced range (*13*–*15*) and is known to induce defenses in *M. edulis* from several populations (*14*, *16*, *17*). Small mussels are vulnerable to both crab species (*12*), show high relative growth amenable to detecting induced defenses, and represent a crucial, prereproductive stage under strong selection.

Given the invasion history of these two crabs, *M. edulis* in northern New England (specifically northeastern Maine) has never experienced predation by *H. sanguineus*. Because the genus *Hemigrapsus* is not native to the Atlantic, neither have they been exposed to any *Hemigrapsus* congeners. However, they have experienced predation by *C. maenas* for more than 50 years. In contrast, mussels in southern New England have experienced predation by *C. maenas* and *H. sanguineus* for 100+ and ~15 years, respectively. To determine whether natural selection has altered the mussels' capacity to respond to these two crabs, we quantified the responses of mussels from these northern and southern populations to these two crab predators. If predator cues are species-specific, and if selection has altered the capacity of mussels to recognize and respond to these invasive predators, we expected that mussels from southern New England would respond to cues from both crabs, whereas northern mussels would respond to cues from *C. maenas* but not *H. sanguineus*.

To compare the inducible defenses of mussels from northern and southern New England in response to *C. maenas* and *H. sanguineus*, we collected mussels (13- to 20-mm shell length) from floating docks at six sites each in northern Maine and southern New England and brought them to Northeastern University's Marine Science Center at Nahant, MA (Fig. 1) (*18*). These mussels were then raised with nonlethal, waterborne cues from *C. maenas*, *H. sanguineus*, or no predator (control). Using the final measurements of each mussel's shell thickness index (STI), adjusted to its initial STI, we assessed the development of inducible defenses (*19*). After 3 months, mussels had grown, and mussels from northern and southern New England had thickened their shells differently in response to waterborne cues from the two invasive crab predators (i.e., there was a significant population by predator treatment interaction) (*20*). Mussels from southern sites thickened their shells in response to waterborne cues from *H. sanguineus* relative to controls ($P = 0.011$), and mussels appeared to thicken their shells in response to *C. maenas*, although the trend was not significant ($P = 0.145$) (Fig. 2). In contrast, although mussels from northern sites developed significantly thicker shells in response to cues from *C. maenas* ($P = 0.001$), they did not respond to cues from *H. sanguineus* ($P = 0.573$) (Fig. 2). In addition, there were clear population differences in the temperature-sensitive process of shell accretion, with mussels from northern populations thickening their shells more than mussels from southern populations (Fig. 2). These findings suggest that northern and southern mussel populations are

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genetically distinct. This pattern of warm water–adapted mollusks secreting shell more slowly than northern conspecifics is consistent with countergradient variation, a pattern seen in the New England snail *Littorina obtusata* (*21*).

To determine whether the previous results were robust or influenced by a laboratory setting more similar to northern collection sites (e.g., water temperature and concentration of background cues from *H. sanguineus*), we ran an additional induction experiment under field conditions more similar to southern sites. We collected another generation of small *M. edulis* from similar northern and southern floating docks (Fig. 1) and raised them for 3 months while exposed to nonlethal, waterborne cues from unfed *C. maenas*, unfed *H. sanguineus*, or no predator (control). In this in situ experiment, mussels and predators were housed in steel mesh cages suspended from a floating dock in Woods Hole, Massachusetts. Mussels were separated from the cue crabs by steel mesh but could also experience any background cues due to ambient crabs in this environment (*19*). These mussels responded to the cue crabs nearly identically to the previous laboratory experiment, with only northern mussels not responding to *H. sanguineus* (Fig. 3) (*22*).

Our results clearly indicate that mussels from populations in northern and southern New England respond differently to waterborne cues from *H. sanguineus*. Yet, mussels in both regions express similar induced shell thickening in response to *C. maenas*, a resident throughout this coast for more than 50 years. Although brief, we believe the historical contact with and predation by *H. sanguineus* accounts for the divergent mussel responses. The mussel's inducible response to *H. sanguineus* reflects natural selection favoring the recognition of this novel predator through rapid evolution of cue specificity or thresholds (*23*). In addition, this response may be brought about by a novel mechanism of shell thickening; however, it more likely relies on mechanisms for induced defenses to other crabs (*8*). Despite the mussel's planktonic larvae, the response to *H. sanguineus* manifested by southern *M. edulis* has not spread to northern mussels. This suggests strong local adaptation and/or mostly unidirectional gene flow due to dispersal barriers such as the predominantly southwestward currents in northern New England (*24*)

Although invasive predatory crabs can induce defenses in native mollusks (*5*, *14*, *16*), these previous examples did not establish that predator recognition and an inducible morphological defense emerged as a result of selection from the invasive predator. Inducible morphological defenses are distinct from other prey defenses (i.e., behavioral responses and fixed traits) because they are often irreversible and they may require a sizeable time lag to develop after predator cues are detected (*25*, *26*). The few examples of natural selection by invasive predators deal with the alteration of existing predator-specific responses, fixed traits, and adaptive behavioral responses (*1*, *3*, *4*, *7*).

Although recent historical contact with *H. sanguineus* appears to have selected for predator recognition in *M. edulis*, we cannot rule out nonheritable processes in individual mussels, such as learning by native prey (*27*) or conditioned predator recognition. However, there are no examples of inducible morphological defenses resulting strictly from learning. In addition, in situ background cues necessary for learning (*28*) appeared to have a negligible effect in our system. At the time of the experiments, *H. sanguineus* was only recently established in Nahant and thus much less abundant compared with southern New England where the crab had been established for several years. If background cues were influential in our system, southern control mussels in the in situ experiment would have thickened their shells, diminishing the difference between control and *H. sanguineus*–exposed mussels in our Woods Hole field experiment relative to the Nahant laboratory experiment. However, this difference was greater in the in situ field experiment than in the Nahant lab experiment, suggesting that ambient background cues were not sufficient to influence our experiments or learning in southern mussels before their collection.

Alternatively, the differing mussel responses to the two crabs may be related to heritable population differences in recognition of *H. sanguineus* unrelated to the introduction of *H. sanguineus*. However, because the genus *Hemigrapsus* is novel to the Atlantic Ocean, there is

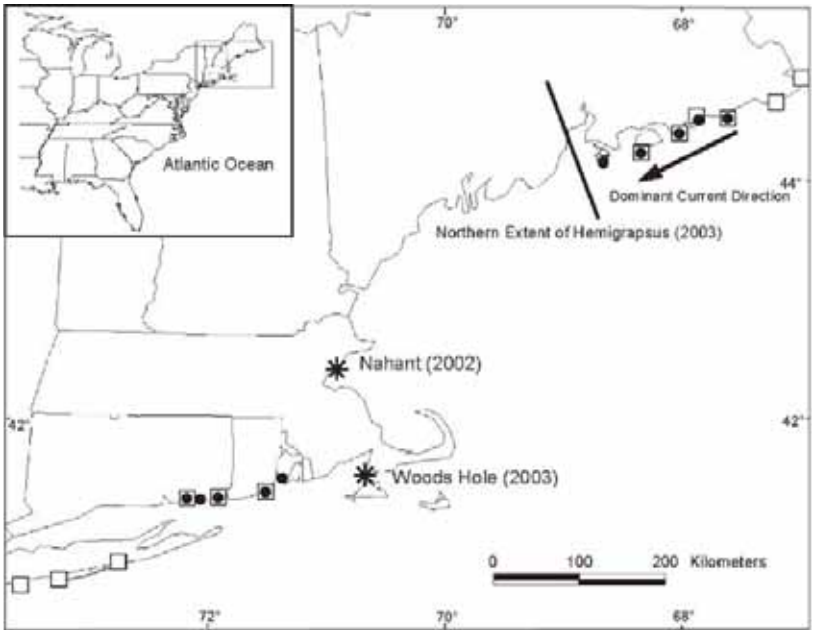

Fig. 1. Sites of the induction experiments at Nahant in 2002 and Woods Hole in 2003 (asterisks). Also indicated are collection sites for mussels used in the Nahant laboratory experiment (open squares) and the Woods Hole field induction experiment (filled circles).

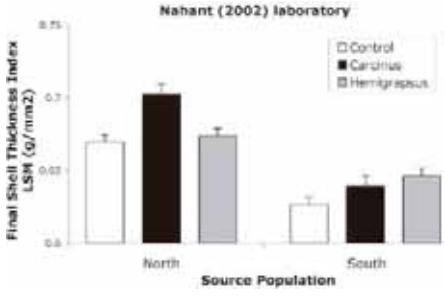

Fig. 2. Adjusted final STI of mussels raised in a laboratory induction experiment at Nahant, Massachusetts, Gulf of Maine. Mussels from northern and southern populations were raised as controls or in the presence of cues from *C. maenas* or *H. sanguineus*. Values are adjusted least square means (LSM) from an analysis of covariance with initial STI as a covariate. Error bars, 1 SEM.

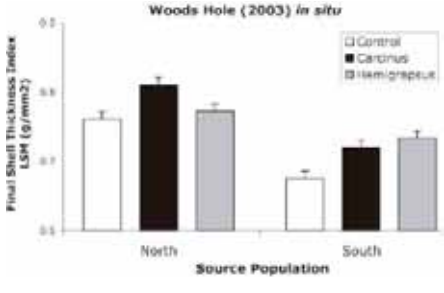

Fig. 3. Adjusted final STI of mussels raised in situ in cages suspended from a floating dock in Woods Hole, MA, in 2003. Mussels from northern and southern populations were raised as controls or in the presence of cues from *C. maenas* or *H. sanguineus*. Values are adjusted least square means (LSM) from an analysis of covariance with initial STI as a covariate. Error bars, 1 SEM.

little reason to believe that any Atlantic mussels recognized it before its invasion. Thus, even if the extremely limited gene flow of *M. edulis* between Europe and North America (*29*) disproportionately influenced northern or southern New England mussels, this effect would not help to explain a population's predisposition to recognize *Hemigrapsus*. Moreover, even if *M. edulis* recognized *H. sanguineus* before its invasion, it is doubtful that the trait would be lost only in northern New England mussels, given the capacity of mussels to maintain cue recognition in the absence of reinforcing predation (*17*). Alternatively, northern New England mollusks may generally experience lower predation than southern conspecifics (*30*). Thus, although previous recognition of *H. sanguineus* per se seems unlikely, southern New England mussels may more readily express inducible defenses to many predator species by responding to a lower threshold of cues or with decreased specificity to predators (*28*). In fact, this potential gradient in cue thresholds and sensitivities may promote the rapid evolution of recognition of a novel, invasive predator in southern New England mussels.

Species interactions can differ on various geographic scales because of local selection and other processes (*31*, *32*). Similarly, there is considerable potential for the evolutionary history of invasive and native species interactions to vary spatially and temporally. Although we have only a nascent understanding of the role of inducible defenses in marine systems (*15*, *33*), this phenomenon is likely highly influenced by the evolutionary history of the interacting species. The confluence of evolutionary and ecological interactions represents an essential field of inquiry to understand fully the impacts of invasive species.

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19. Details are available in Materials and Methods as supporting material on *Science* Online.
20. Analysis of covariance (ANCOVA) of final STI in laboratory experiment at Nahant, MA (2002): Site(Population) $P < 0.0001$; Predator $P = 0.0033$; Population $P = 0.0207$; Predator × Population $P = 0.0249$; Predator × Site(Population) $P = 0.3378$; Initial STI $P < 0.0001$. See table S2 in supporting material on *Science* Online.
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Supporting Online Material

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References

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Loss of a Harvested Fish Species Disrupts Carbon Flow in a Diverse Tropical River

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Harvesting threatens many vertebrate species, yet few whole-system manipulations have been conducted to predict the consequences of vertebrate losses on ecosystem function. Here, we show that a harvested migratory detrital-feeding fish (Prochilodontidae: *Prochilodus mariae*) modulates carbon flow and ecosystem metabolism. Natural declines in and experimental removal of *Prochilodus* decreased downstream transport of organic carbon and increased primary production and respiration. Thus, besides its economic value, *Prochilodus* is a critical ecological component of South American rivers. Lack of functional redundancy for this species highlights the importance of individual species and, contrary to theory, suggests that losing one species from lower trophic levels can affect ecosystem functioning even in species-rich ecosystems.

Widespread interest in the importance of species to ecosystem functioning stems from concerns that the rapid rate of human-induced species losses could affect ecosystem properties and services negatively (*1*). Freshwater ecosystems provide essential ecosystem services and contain a large fraction of species diversity that may be declining faster than the diversity in marine or terrestrial ecosystems (*2*). Humans have overharvested many of the large, long-lived predatory fishes and are now shifting fishing efforts to the abundant, higher-yielding species at lower trophic levels, such as detritivores (*3*). Detritus is the major pathway of energy and material flow in most ecosystems, supports higher trophic levels, and is a major source of inorganic nutrient regeneration and uptake; losses of detritivores could disrupt ecosystem functioning (*4*). Both greater abundance and higher species richness at lower trophic levels

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are assumed to increase functional redundancy (*5*), but these characteristics also make species like detritivores targets for harvesting. Despite being a large percentage of the fish biomass and catch, manipulations of detritivores are less common than those of predators in freshwater and marine ecosystems.

We investigated how the loss of a dominant migratory detritivorous fish, the flannelmouth characin, *Prochilodus mariae*, alters ecosystem metabolism and organic carbon flow in an Andean piedmont river located in the Orinoco basin (Rio Las Marías, 9°10′N, 69°44′W; 225 m elevation; 331 km^2 watershed area; 2002 dry season ranges: 0.142 to 0.782 $m^3\ s^{-1}$ discharge, 10.1 to 20.5 m wetted width). Piedmont rivers supply the Rio Orinoco with 25 to 90% of its inorganic nutrients and particulate organic carbon (POC) (*6*). These rivers support a high diversity of fishes (*7*), with at least 80 species in a 3-km-long segment of Rio Las Marías (*8*), a fourth-order tributary of the Rio Portuguesa that flows into the Rio Apure. Fish diversity in piedmont streams is dominated by omnivorous tetras (Characidae) and insectivorous catfishes (Heptapteridae), few of which are harvested because of their small body size. In contrast, detritivores, such as prochilodontids, constitute 50 to 80% of the fish biomass and catch in the Orinoco and Amazon basins (*7*, *9*) and are declining throughout the Andean Piedmont (*10*). Although there are other common benthic feeders in Andean piedmont streams that consume benthic algae and particulate matter [e.g., *Parodon apolinari* (Parodontidae) and armored catfishes *Ancistrus triradiatus* and *Chaetostoma milesi* (Loricariidae)], they do not reach the biomass of prochilodontids, which are consistently the dominant fish in Rio Las Marías and other South American rivers (*10*–*12*). Dams, deforestation, and pollution threaten prochilodontid populations (*3*, *10*), making experimental tests of their removal relevant to current human impacts.

Prochilodus migrates into Andean piedmont rivers to feed during the dry season (January to April) and spawn while returning to floodplains during the wet season (May to December) (*10*). *Prochilodus* migrations represent a potentially important linkage within river networks, because, by bioturbating, consuming, and egesting large volumes of detritus, this fish may enhance the downstream transport and transformation of materials at a time when hydrologic transport is reduced and algal and bacterial productivities are high in neotropical rivers (*6*, *13*). Replicated small-scale (4-m^2) caging experiments showed that *Prochilodus* decreased benthic particulate matter and changed the composition of microbial biofilms from sediment-dwelling diatoms and heterotrophic bacteria to attached nitrogen-fixing cyanobacteria (*8*, *14*). In addition to their abundance, the effects of *Prochilodus* may be unique, because, by removing particles that reduce light essential for N fixers, they may facilitate a source of primary production that is independent of N limitation (*15*). These results provided the basis for the larger-scale manipulations and longer-term observations reported here.

The downstream flux of POC was positively associated with *Prochilodus* biomass over 6 years [r = 0.76, P = 0.04, d.f. (degrees of freedom) = 4] (Fig. 1) (*16*). In contrast, interannual variation in discharge and biomass of other fishes were not significantly correlated with POC flux ($P > 0.50$) (fig. S3). Hence, relative to other physical and biological factors, fluctuations in the biomass of *Prochilodus* strongly regulated whole-stream transport of POC.

To test the effects of losing *Prochilodus* on carbon flow and metabolism, we used a large-scale experiment in which we selectively removed this single species from the natural ecosystem and left the remaining fish assemblage intact (*16*). The experiment was per-

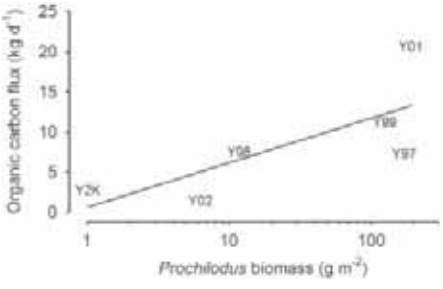

Fig. 1. Interannual variation in organic carbon flux. Whole-stream flux of suspended particulate organic carbon increased as a function of *Prochilodus* biomass (as wet mass). Y97 indicates 1997 data; Y98, 1998 data; etc. Y2K, 2000 data.

Fig. 2. Photographs of the split-stream removal experiment. (**Top**) The plastic divider and 210 m section of Rio Las Marías. (**Bottom**) Visual differences in benthic particulate matter after removing *P. mariae* (right) compared with the intact fish assemblage (left).

formed in a riffle-run-pool segment of river by installing a 210-m barrier down the center of the river and removing *Prochilodus* from one side (*16*). The split-stream experiment allowed us to measure the effects of a wide-ranging consumer on ecosystem processes that occur at large spatial scales and in the presence of other naturally varying biotic and abiotic processes. We measured whole-stream primary production and respiration of organic carbon by using the open-channel diel-oxygen change method (*16*). We also measured the downstream flux and the benthic biomass of POC and calculated organic carbon turnover length (*16*, *17*), the average distance an organic carbon molecule travels before being respired.

Removing *Prochilodus* increased benthic particulate matter on the stream bottom (Fig. 2B) and altered multiple components of organic carbon flow (Fig. 3). Impacts of removing *Prochilodus* on carbon flow equaled or exceeded effects of removing all fish (*18*), invertebrates (*19*), shrimps (*20*), and predatory fish in other streams and lakes (*21*–*23*). The biomass of POC on the streambed increased 450% (Fig. 3, A and B) after *Prochilodus* removal, a result consistent with replicated small-scale experiments demonstrating that *Prochilodus* effects occurred within 48 hours and persisted for at least 40 days during the 3-month dry season (*14*). The downstream flux of suspended POC decreased by 60% immediately after *Prochilodus* removal (Fig. 3, C and D) because of decreased bioturbation, consumption, egestion, and selective sorting of benthic POC by *Prochilodus* [Supporting Online Material (SOM) Text]. The time it takes POC to travel a given distance downstream is a measure of its retention. Before the manipulation, the residence time of POC per longitudinal meter of river (*16*, *17*) was similar between the reference (mean ± 1 SD = 0.43 ± 0.09 day m^{-1}) and the treatment (mean ± 1 SD = 1.5 ± 0.54 day m^{-1}) but increased by an order of magnitude, 0.8 ± 0.19 day m^{-1} in the reference compared with 10.91 ± 3.67 day m^{-1} in the treatment, after removing *Prochilodus* ($t_{3.01}$ = 5.27, P = 0.01). Thus, during the dry season when floods are small and infrequent, *Prochilodus* enhances the transport of POC, which is a source of energy to downstream communities and a key biogeochemical function of rivers (*24*).

Because benthic POC and biofilms increased after removing *Prochilodus*, heterotrophic respiration (other than by *Prochilodus*) increased by 200% in the treatment (Fig. 3, E and F). In addition, gross primary production (GPP) doubled after *Prochilodus* removal (Fig. 3, G and H). The percent increase in community respiration (CR, equal to autotrophic plus heterotrophic respiration) was greater than the percent increase in GPP; therefore, the ratio of production to respiration (P:R) decreased by 150% after *Prochilodus* removal (Fig. 3, I and J). Similarly, the deficit in net ecosystem metabolism (NEM = GPP – |CR|) was 42% greater after removing *Prochilodus* (Fig. 3, K and L). Thus, removing *Prochilodus* decreased the proportion and the absolute amount of CR supported by current autotrophic production. Consequently, without *Prochilodus*, river food webs may be supported by organic carbon produced earlier or imported from upstream and the terrestrial ecosystem rather than by current, local autotrophic production.

Organic carbon turnover length, or the downstream distance an organic carbon molecule travels until metabolized, is a measure of the longitudinal scale at which downstream ecosystems and food webs are linked to those upstream (*17*). Nutrient spiraling theory predicts consumers should increase turnover length by decreasing the benthic bacterial biomass and increasing the downstream flux of particles (*17*, *25*). Consistent with this theory, removal of *Prochilodus* decreased turnover length by 35%, from 1.0 to 0.65 km (Fig. 3, M and N). With *Prochilodus* present, the coupling of materials and energy from upstream to downstream was enhanced. Hence, the loss of *Prochilodus* decreased the

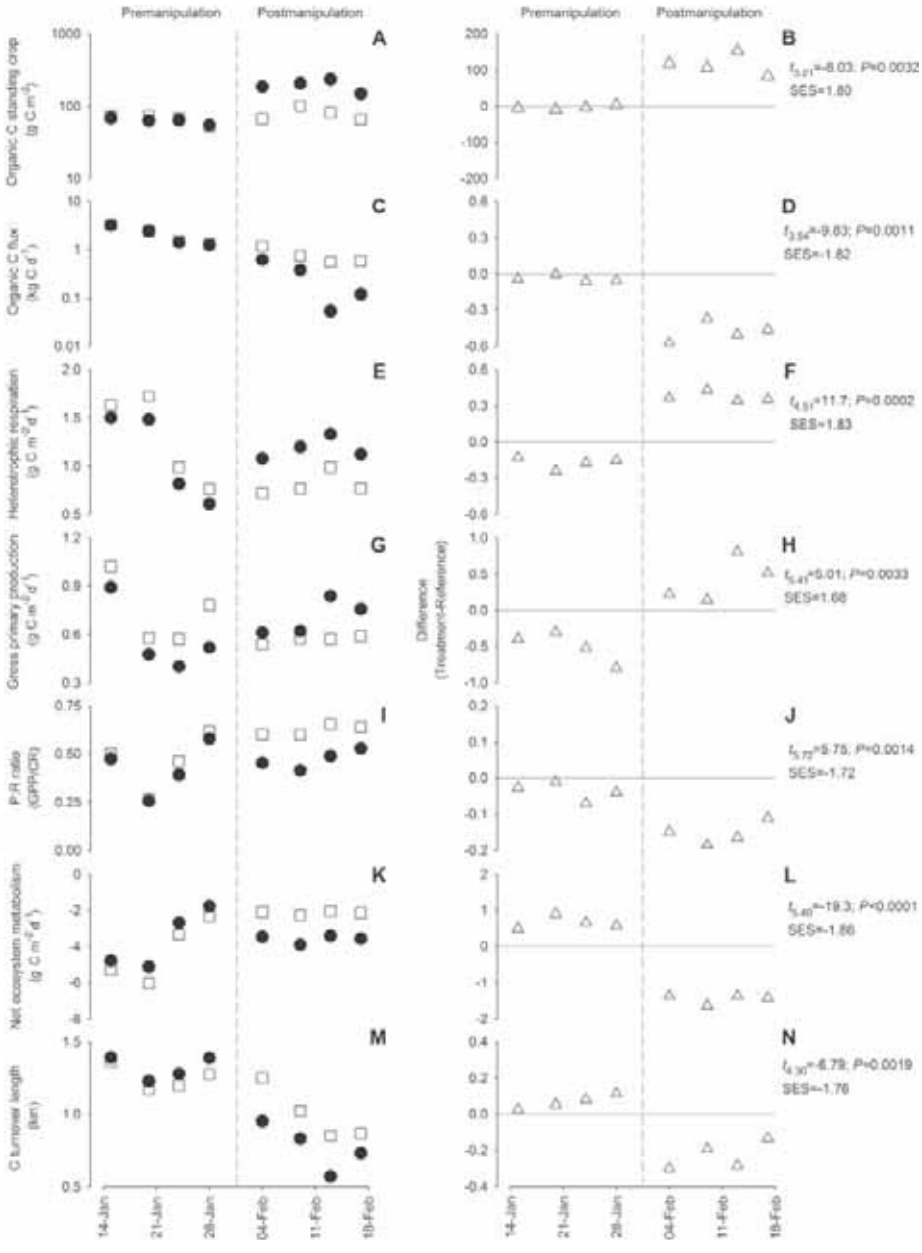

Fig. 3. Ecosystem properties in the treatment and reference area of the split-stream experiment before and after removal of *P. mariae*. (**A** to **M**) Measured values in the treatment (solid circles) and the reference (open squares). (**B** to **N**) Differences between measured values of the treatment and the reference. *Prochilodus* was selectively removed on 31 January 2002 (vertical dashed line) from the treatment. Note the logarithmic *y* axis. The *t* and *P* values were calculated by using the Welch-Satterthwaite-Aspin *t* test, and SES is the standardized effect size.

spatial scale of organic carbon availability, and the metabolism of organic carbon was more localized during the dry season, a time when hydrologic transport is low.

Given that the removal of *Prochilodus* altered ecosystem function, we investigated the effects of human harvesting on *Prochilodus* body size, a determinant of reproductive success and a proxy for changes in population size due to overharvesting (*3*). We evaluated long-term data on body mass of field and museum specimens of *Prochilodus* collected throughout the Orinoco basin from 1978 to 2004 (*16*).

Prochilodus body mass has declined substantially during the past 25 years (Fig. 4A), which we attribute to removal of larger individuals by net-based fishing. The mean maximum body mass decreased from 856 to 201 g, an initial rate of decline of 19 ± 9.1% per year ($t_{22} = -1.80$, $P = 0.03$). The current mean maximum body mass of 201 ± 81 g ($t_{22} = 2.50$, $P = 0.01$) is 20% below the mean size at which females become reproductively mature (*10*) and may represent a refugium body mass caused by size-selective harvesting. Concurrently, fishermen have decreased the mesh size of their nets. By making their own cast nets using their fingers to gauge the mesh size, fishermen have decreased their net mesh size from four to two finger widths over the past 25 years (*26*), a numerical decrease from 6 to 3 cm (Fig. 4B). Hence, the body depth (greatest dorsal-ventral length) of refugium-sized *Prochilodus* is now 3 to 3.5 cm (*16*). Decreasing net mesh size and body mass are hallmarks of overfishing and are correlated with decreasing fish population size (*3*). Decreasing body size may also change pathways of carbon flow, because fish consumption rates generally decrease with decreasing body size. Thus, size-selective harvesting may have long-lasting negative feedbacks on fish populations, ecosystem function, and the flow of protein to humans and other animals, eroding an important ecosystem service (*1*, *3*).

These results have several implications for conservation management and our understanding of ecosystem function. First, the results show low functional redundancy in a diverse ecosystem for a single detritivorous fish species that regulates fundamental ecosystem processes, synthesis and degradation of organic carbon. This finding contradicts the prediction that more individuals and species at lower trophic levels impart a degree of insurance against changes in ecosystem functioning (*5*). Furthermore, in rivers where *Prochilodus* migrations have been permanently blocked, compensatory responses by other fishes have not occurred (*10*). Second, these results are not restricted to spatially localized, short-term processes. POC accumulated on the streambed may eventually be transported downstream during wet season floods; however, most POC transported by floods may not be available or used by many organisms because it is pulsed so rapidly through downstream areas. Moreover, dry season floods are rare or small in magnitude in the Andean Piedmont, so it is unlikely these events would remove much POC or reduce the effects of *Prochilodus* (fig. S4). Thus, *Prochilodus* reduces the spatial and temporal variability of organic carbon flow, resulting in a more constant supply of energy and materials, especially during the dry season when detrital resources are scarce and fish growth is low (*7*, *27*). Lastly, the results show the potential ramifications to ecosystem-level carbon flow of losing a species that is currently harvested by humans. Considering the effects we observed in 2002 when *Prochilodus* biomass was low, we suspect that these effects may be even greater in other years or in other piedmont rivers with higher *Prochilodus* biomass. In many ecosystems, we know which species or functional groups are threatened by human activities, and selective experimental removals of species targeted by humans could be informative for predicting whether their losses will change ecosystem functioning substantially, especially if traits selected by humans covary with those that enhance species impacts (*28*).

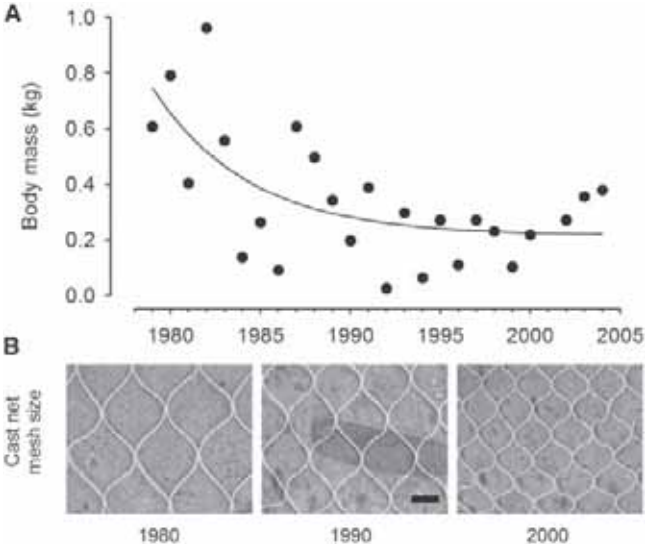

Fig. 4. Time trends of body mass for the migratory fish *P. mariae*. (**A**) Mean maximum body mass of individuals in the upper quartile for specimens collected throughout the Orinoco basin. The equation is *Prochilodus* mean maximum body wet mass (g) = $0.214 + e^{-0.19t}(0.856 - 0.214)$, which fit better ($F_{1,22} = 5.359$, $P = 0.03$) than a simpler, semi-log-linear model (lack-of-fit test: $F_{1,22} = 66.596$, $P < 0.0001$). (**B**) Photographs of cast nets made over the past 3 decades by a fisherman in the community near the study site. Scale bar indicates 2.5 cm.

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Supporting Online Material

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Polarized Light Cues Underlie Compass Calibration in Migratory Songbirds

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Migratory songbirds use the geomagnetic field, stars, the Sun, and polarized light patterns to determine their migratory direction. To prevent navigational errors, it is necessary to calibrate all of these compass systems to a common reference. We show that migratory Savannah sparrows use polarized light cues from the region of sky near the horizon to recalibrate the magnetic compass at both sunrise and sunset. We suggest that skylight polarization patterns are used to derive an absolute (i.e., geographic) directional system that provides the primary calibration reference for all of the compasses of migratory songbirds.

Studies of migratory songbirds provide evidence that they use multiple compasses (*1*–*5*), but the integration of these multimodal directional cues is poorly understood. Although many species of songbirds have been shown to have an innate sense of migratory direction derived from magnetic (*6*–*8*) and/or celestial (*9*–*11*) cues, these compass systems are "recalibrated" when exposed to conflicting directional information. Such conflicts occur naturally when magnetic declination changes; this change is especially pronounced at high latitudes (*12*). When cue availability changes with time of day and/or weather conditions, avoiding navigational errors requires that information from all of the compasses be calibrated with respect to a common reference (*13*). Here, we show that the primary calibration reference is derived from horizon-polarized light cues at sunrise (SR) and sunset (SS).

Research on the integration of compass information by migratory songbirds has focused on experiments in which birds were given conflicting directional information from two or more cues to determine which of the cues is given greater saliency and whether the conflict results in recalibration of one or more of the compass systems. Such experiments have produced variable and sometimes contradictory findings. Most studies suggest that birds give precedence to celestial light cues and use these cues to recalibrate the magnetic compass before the onset of migration (*6*, *7*, *14*–*16*) but that the reverse is true once birds begin migration; that is, the magnetic compass takes precedence over and is used to calibrate celestial light cues (*17*–*19*). However, two studies demonstrated recalibration of the magnetic compass with respect to SS cues during migration (i.e., the cue hierarchy normally observed during the premigratory period) (*20*, *21*). In a recent review (*13*), we found that recalibration of the magnetic compass with respect to SS (or SR) cues occurred during both the premigratory and migratory periods when birds exposed to conflicting information were able to see celestial polarized light cues from the horizon sky. In studies carried out during migration that failed to show magnetic compass recalibration, birds were exposed to cue conflicts in orientation cages/funnels that blocked a view of the sky near the horizon (*13*). When deprived of polarized light cues from this region of sky, birds gave precedence to magnetic cues and secondarily calibrated other celestial cues (e.g., star patterns and/or overhead polarized light cues) with respect to the magnetic field (*22*–*29*).

To clarify the role of polarized light cues in calibration of the magnetic compass, we tested whether wild-caught Savannah sparrows, *Passerculus sandwichensis*, recalibrated their magnetic compass when exposed to conflicting magnetic and polarized light cues near the horizon at SR or SS. We captured juvenile and adult birds in the Yukon Delta National Wildlife Refuge, Alaska, during autumn 2005. The birds were held indoors under the natural photoperiod without access to natural visual cues (*30*). All orientation experiments were started at around SS and carried out indoors in the ambient magnetic field in the absence of celestial cues, thus requiring the birds to use their magnetic compass for orientation (*30*). The magnetic orientation of individual birds selected for experimental exposures is shown in Fig. 1B (see also table S1). They were given a single exposure for 60 min around SR or SS to a cue conflict between the ambient magnetic field and an artificial polarization pattern rotated by ±90° relative to the natural polarization pattern at that time of day (Fig. 1C). During exposure, the birds had a full view of the surroundings, including the horizon, through the polarization filters that produced the artificial pattern (*30*).

After exposure to the cue conflict, the magnetic compass orientation of the birds was again tested indoors. The distribution of bearings was indistinguishable from random (SR, mean bearing or axis $\alpha = 138°/318°$, $r = 0.08$, $P = 0.82$, $N = 30$; SS, $\alpha = 309°$, $r = 0.17$, $P = 0.59$, $N = 20$; Fig. 1D and table S1) and was significantly different from the birds' initial responses (Fig. 1B; nonparametric two-sample Watson U^2 test: SR, $U^2 = 0.33$, $P < 0.005$; SS, $U^2 = 0.42$, $P < 0.001$). The absence of significant clustering of bearings after cue-conflict exposure suggests that the birds did not orient in a consistent direction or axis relative to the magnetic field. Thus, the birds had not calibrated the magnetic compass in a fixed relationship (e.g., perpendicular or parallel) to the polarization pattern or to the Sun's position, which was visible to some birds during the cue-conflict exposure (Fig. 1, D and E, open symbols; table S1). However, when each bird's response after cue-conflict exposure (Fig. 1D) was plotted as a deviation from its initial response (Fig. 1B), the deviations were bimodally distributed along an axis perpendicular to their earlier response (SR, $\alpha = 85°/265°$, $r = 0.54$, $P < 0.001$, $N = 30$; SS, $\alpha = 94°/274°$, $r = 0.58$, $P < 0.001$, $N = 20$; Fig. 1E and table S1).

These findings indicate that the birds had shifted their orientation relative to the magnetic field by ±90°, corresponding to the rotation of the artificial polarization pattern relative to the natural pattern at the same time of day (i.e., SR or SS; Fig. 1E). The alternative hypothesis that calibration of the magnetic compass occurs only at SS or only at SR is excluded by the data (Fig. 1E, triangles outside circle) (*30*). Moreover, both juvenile and adult birds recalibrated their magnetic compass (adult birds were exposed and tested only at SR) (*30*) (table S1). Birds that recalibrated the magnetic compass at SR subsequently did so again at SS, and vice versa (*30*) (table S1).

A small sample of birds exposed to a rotated polarization axis that did not include the horizon exhibited orientation that was indistinguishable from their responses before exposure, indicating that the magnetic compass had not been recalibrated (fig. S1E and table S2) (*30*).

Our findings support the following conclusions: (i) The magnetic compass is recalibrated with respect to polarized light cues at both SR and SS; a conflict between magnetic and polarized light cues at either time of day resulted in recalibration of the magnetic compass. (ii) This recalibration occurs both before [as shown by previous investigators, reviewed in (*13*)] and during migration, and in both juvenile and adult birds. (iii) A view of the polarization patterns from the sky near the horizon is required for magnetic compass recalibration. Thus, the failure to observe magnetic compass recalibration in many studies carried out during migration is probably the result of exposure to the cue conflict in cages/funnels that obscured the natural horizon (*13*). In conjunction with earlier work showing that Sun and star compass calibrations are secondarily derived from magnetic and polarized light cues (*23*, *24*, *31*), these

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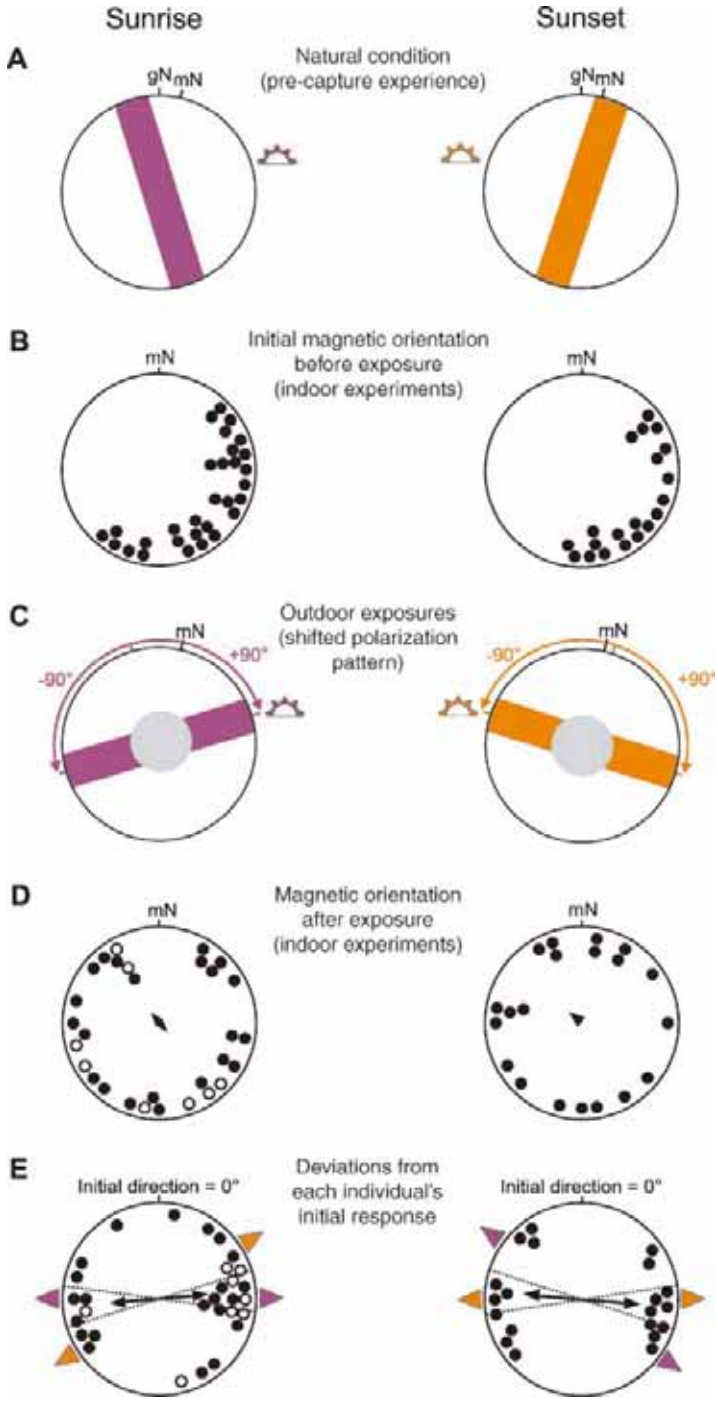

Fig. 1. Magnetic orientation of Savannah sparrows exposed to polarization pattern shifted ±90° at sunrise (SR; left) and sunset (SS; right). (**A** and **C**) 360° view of sky under natural and experimental conditions (gN, geographic north; mN, magnetic north). Purple and orange bars indicate mean position of band of maximum polarization (BMP) at SR and SS, respectively; gray zones indicate areas of sky not visible during exposure. (A) Natural relationship between SR/SS celestial cues and geomagnetic field. (C) Alignment of ±90° shifted polarization axis during exposure to cue conflict. (**B** and **D**) Magnetic orientation of birds tested indoors, plotted relative to mN = 0°. Open symbols denote birds for which the disk of the Sun was visible during exposure. Arrowheads show mean bearing or axis; length (measure of concentration) is drawn relative to the radius of the circle = 1. (B) Orientation of birds selected for exposure; (D) magnetic orientation after exposure. (**E**) Deviations from each individual's initial response before exposure [initial direction (B) of each individual set to 0°]. Arrows show mean bearing or axis; dashed lines give 95% confidence intervals (*38*); triangles outside circles give predicted responses for a ±90° shift in BMP relative to the natural SR (purple) or SS (orange) position. See (*30*) and table S1.

findings suggest that horizon polarization patterns at SR and SS provide the primary calibration reference for all the compass systems of migratory songbirds.

At SR and SS, the band of maximum polarization (BMP) passes directly through the zenith (*32*, *33*) and, along with the e-vector (electrical vector) of polarized light, is aligned vertically on the horizon (*30*) (fig. S2, A and B). In contrast to Sun position, therefore, the intersections of the BMP with the horizon at SR and SS are independent of topography (i.e., horizon height). In addition, because the BMP and e-vector are vertically aligned only at SR and SS, their use as a calibration reference would not require a time compensation mechanism (*34*). Averaging the intersections of the BMP with the horizon during a successive SR and SS would enable migratory birds to derive an absolute reference system that is "fixed" with respect to the north-south meridian at any location on Earth and thus independent of latitude and time of year (*30*, *34*) (fig. S2C). Periodic updating of the relationship between the polarization patterns at SR and SS (i.e., their angular "split" on either side of the meridian) would make it possible to use either the SR or SS pattern to estimate the reference direction and calibrate other compass systems (*30*) (fig. S2, D and E).

Changes in latitude and time of year produce opposite shifts in the alignments of the BMP at SR and SS (fig. S3). Consequently, use of the polarization pattern at either SR or SS alone as an independent calibration reference (i.e., without averaging) can result in a gradually curving migratory route that may under some conditions be adaptive (*30*) (fig. S3). However, such routes depend on the timing of migration and would therefore be altered by delays such as those caused by extended periods of inclement weather.

In species like our Savannah sparrows that use both SR- and SS-polarized light patterns (Fig. 1), failure to integrate the information from these two times of day would produce an unpredictable "zig-zagging" migratory path depending on whether the clear skies necessary to see the polarization pattern occurred most recently at SR or at SS (*30*). Thus, not only does averaging of SR- and SS-polarized light cues provide a calibration reference that is unaffected by changes in latitude and time of year, but failure to do so would decrease the accuracy and increase the distance of migration. In species that use both SR- and SS-polarized light cues to calibrate other compass systems, therefore, both curving migratory routes and abrupt changes in migratory direction associated with major topographic features (such as oceans and mountain ranges) are likely to involve secondary adaptations rather than properties of the underlying calibration reference system (*12*, *30*, *35*–*37*).

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 and S2
References

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Requirement for Coronin 1 in T Lymphocyte Trafficking and Cellular Homeostasis

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The evolutionarily conserved actin-related protein (Arp2/3) complex is a key component of actin filament networks that is dynamically regulated by nucleation-promoting and inhibitory factors. Although much is known about actin assembly, the physiologic functions of inhibitory proteins are unclear. We generated *coronin 1*$^{-/-}$ mice and found that coronin 1 exerted an inhibitory effect on cellular steady-state F-actin formation via an Arp2/3-dependent mechanism. Whereas coronin 1 was required for chemokine-mediated migration, it was dispensable for T cell antigen receptor functions in T cells. Moreover, actin dynamics, through a mitochondrial pathway, was linked to lymphocyte homeostasis.

The integrity of the actin cytoskeletal network is critical for a diverse range of biological processes and is dynamically regulated by a cohort of actin-associated proteins. The Wiskott-Aldrich syndrome (WAS) and the suppressor of cyclic adenosine monophosphate (cAMP) receptor (SCAR) proteins promote actin nucleation and assembly via the Arp2/3 complex (*1*–*3*), whereas inhibitory proteins, which include coronin, tropomysosin, and caldesmon, oppose Arp2/3 function (*4*–*6*). The evolutionarily conserved coronin family of actin-binding proteins has been implicated in the regulation of multiple actin-mediated cellular functions, including cell migration, cytokinesis, and cell growth of *Dictyostelium discoideum* and *Saccharomyces cerevisiae* (*7*–*12*). Among the seven mammalian coronin family members, coronin 1 (also known as coro1a, TACO, or p57) is preferentially expressed in cells of hematopoietic origin, where it is coexpressed with other more widely expressed coronin family members that include coronins 2, 3, and 7 (fig. S1A) (*13*). In mammals, coronin 1 colocalizes with F-actin surrounding phagocytic vesicles in neutrophils and macrophages and F-actin–rich membranes in activated T cells (*14*–*16*).

To investigate the physiological role of coronin 1, we generated *coronin 1*$^{-/-}$ mice (fig. S1, B and C). No coronin 1 protein was detected in thymocytes, splenocytes, or bone marrow–derived cells isolated from *coronin 1*$^{-/-}$ mice, and expression of coronins 2 and 3 was not altered (fig. S1D). Analysis of lymphoid tissues revealed normal segregation of T and B cells but a paucity of T cells in spleens and lymph nodes of *coronin 1*$^{-/-}$ mice (Fig. 1A). Both CD4$^+$ and CD8$^+$ T cells were decreased in the blood, spleen, and lymph nodes (Fig. 1B). Naïve, but not memory/effector, splenic T cells were decreased, although both were reduced in the blood and lymph nodes of *coronin 1*$^{-/-}$ mice. Thymic cellularity and subpopulations were similar between *coronin 1*$^{-/-}$ and *coronin 1*$^{+/+}$ mice, although a small reduction in mature CD4$^+$ and CD8$^+$ (CD69$^-$) *coronin 1*$^{-/-}$ thymocytes was observed (Fig. 1C and fig. S1, I to J). An analysis of *coronin 1*$^{-/-}$ mice bearing either major histocompatibility complex (MHC) class 1 restricted H-Y or class II restricted DO11.10 transgenic T cell antigen receptors (TCRs) revealed normal thymic development and decreased naïve T cells in lymph nodes (Fig. 1D).

The requirement for coronin in cell motility in *D. discoideum* prompted us to examine whether coronin 1 may play a role in thymic emigration and homing to secondary lymphoid organs. CD4$^+$ *coronin 1*$^{-/-}$ thymocytes demonstrated reduced spontaneous migration and transwell migration to CCL19, CXCL12, and CCL25 (Fig. 2A). Defects in chemotaxis were also observed in splenic CD4$^+$ naïve and effector/memory *coronin 1*$^{-/-}$ T cells (fig. S2B). *Coronin 1*$^{-/-}$ T cells also demonstrated compromised migration in whole-organ thymic cultures and in vivo thymic egress (fig. S2, C and D). Lastly, adoptive transfer of differentially labeled *coronin 1*$^{-/-}$ and *coronin 1*$^{+/+}$ CD4$^+$ thymocytes revealed ~60% decreased homing of *coronin 1*$^{-/-}$ cells to lymph nodes (Fig. 3A). Thus, coronin 1 plays important functional roles in cell motility and chemokine-mediated homing of T lymphocytes to secondary lymphocyte organs.

Because the actin cytoskeleton is required for cellular polarization and lymphocyte migration, we analyzed the morphologic changes induced by CCL19. Whereas stimulated *coronin 1*$^{+/+}$ T cells acquired a polarized phenotype with unipolar accumulation of talin beneath the cell membrane opposite of the uropod, *coronin 1*$^{-/-}$ T cells failed to develop a uropod and formed multiple patch-like talin-rich

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clusters that were distributed irregularly around the cell cortex (Fig. 2B). Notably, *coronin 1*$^{-/-}$ T cells had increased amounts of F-actin. Despite enhanced basal F-actin formation, *coronin 1*$^{-/-}$ naïve T cells demonstrated lower degrees of CCL19-induced F-actin formation (fig. S3A). These cytoskeletal defects were associated with a selective defect in SDF1α-mediated Rac1 activation but not activation of Erk, Akt, or Rsk signaling pathways (Fig. 2C and fig. S3B). Although cytoskeletal reorganization is also requisite for TCR function, *coronin 1*$^{-/-}$ DO11.10 TCR^{+} T cells demonstrated normal antigen-induced proliferation and accumulated F-actin at the T cell–antigen-presenting cell (APC) interface (fig. S4, A to C). Thus, *coronin 1* plays a critical role in chemokine-regulated uropod formation, talin polarization, and migration but is dispensable for TCR-mediated functions.

Although adoptively transferred *coronin 1*$^{-/-}$ T cells were compromised in their ability to migrate to lymph nodes, no compensatory increase of *coronin 1*$^{-/-}$ T cells was observed in the circulation (Fig. 3A), which suggested that loss of coronin 1 may also compromise cell survival. Correspondingly, *coronin 1*$^{-/-}$ mice had a higher percentage of annexin V^{+} CD4^{+} and CD8^{+} thymocytes, particularly in mature CD69^{lo} cells (Fig. 3B). A similar enhancement in annexin V^{+} cells was observed in peripheral naïve T cells. This increased rate of in vivo apoptosis was cell intrinsic, because in vitro incubation of *coronin 1*$^{-/-}$ naïve T cells exhibited greater spontaneous cell death compared with *coronin 1*$^{+/+}$ T cells (Fig. 3C). Increased apoptosis was associated with cleavage of caspases 3 and 9 and reversed by caspase inhibitors (Fig. 3, D and E). In addition, *coronin 1*$^{-/-}$ T cells demonstrated increased spontaneous release of cytochrome c to the cytoplasm (Fig. 3F). In contrast, effector/memory T and splenic B cells did not demonstrate any increase in spontaneous apoptosis (fig. S5B). Thus, coronin 1 is required for naïve T cell survival.

The linkage of actin dynamics to mitochondrial membrane potential (MMP), caspase activation, and cellular viability in yeast (*17*) and

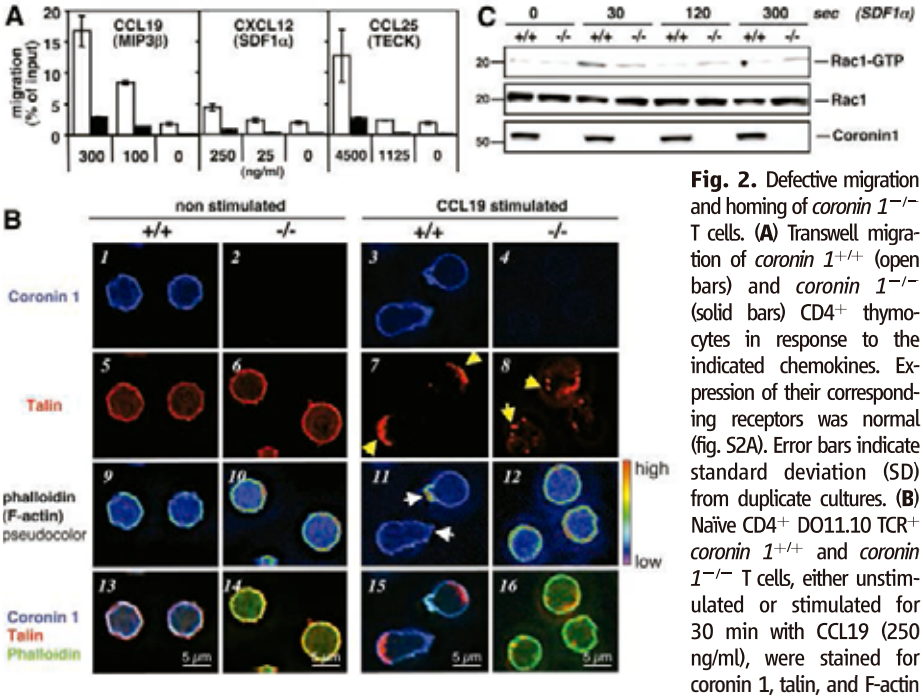

Fig. 2. Defective migration and homing of *coronin 1*$^{-/-}$ T cells. (**A**) Transwell migration of *coronin 1*$^{+/+}$ (open bars) and *coronin 1*$^{-/-}$ (solid bars) CD4^{+} thymocytes in response to the indicated chemokines. Expression of their corresponding receptors was normal (fig. S2A). Error bars indicate standard deviation (SD) from duplicate cultures. (**B**) Naïve CD4^{+} DO11.10 TCR^{+} *coronin 1*$^{+/+}$ and *coronin 1*$^{-/-}$ T cells, either unstimulated or stimulated for 30 min with CCL19 (250 ng/ml), were stained for coronin 1, talin, and F-actin (phalloidin) and analyzed by deconvolution microscopy (DeltaVision, RT, Applied Precision, LLC, Issaquah, WA). Yellow arrows point to talin clusters, and white arrows indicate the position of uropod formation. Similar to the dysregulated talin staining, phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P_3] also accumulated asymmetrically in CCL19-stimulated *coronin 1*$^{-/-}$ T cells (fig. S3C). (**C**) DO11.10 TCR *coronin 1*$^{+/+}$ and *coronin 1*$^{-/-}$ T cells, stimulated with CXCL12 for the indicated times, were analyzed for Rac activation (top). Cell extracts were also subjected to immunoblotting with Rac1 and coronin 1 monoclonal antibodies (mAbs). GTP, guanosine 5′-triphosphate.

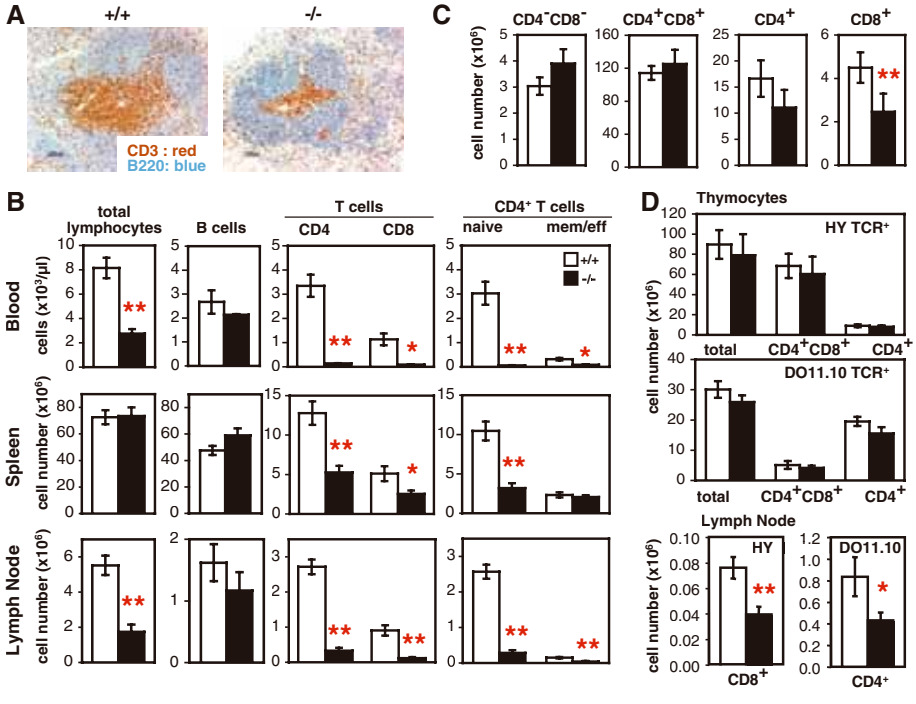

Fig. 1. Reduced numbers of peripheral T cells in *coronin 1*$^{-/-}$ mice. (**A**) Immunohistochemical staining for CD3 (red) and B220 (blue) of splenic tissue sections from *coronin 1*$^{+/+}$ and *coronin 1*$^{-/-}$ mice. (**B**) Total lymphocyte, B220^{+} B cells, CD4^{+} and CD8^{+} T cells, and CD4^{+} naïve (CD44^{lo}CD62L^{hi}) and effector/memory (CD44^{hi}CD62L^{lo}) T cell subset numbers in blood (n = 4), spleen (n = 8), and inguinal lymph nodes (n = 8) were quantified from *coronin 1*$^{+/+}$ (open bars) and *coronin 1*$^{-/-}$ (solid bars) mice (age 5 to 7 weeks). Statistical analysis was performed by two-tailed Student's t test (*P < 0.05, **P < 0.01) for all figures. Error bars indicate the standard error of the mean (SEM). Representative FACS profiles are shown in fig. S1E. The only discernible non–T cell defect was decreased circulating eosinophils (table S1 and fig. S1, F to H). (**C**) Thymocyte subsets were quantitated from *coronin 1*$^{+/+}$ (open bars) and *coronin 1*$^{-/-}$ (closed bars) mice (n = 12). Representative FACS profiles are shown in fig. S1F. (**D**) Thymocytes and lymph node cells from female *coronin 1*$^{+/+}$ and *coronin 1*$^{-/-}$ H-Y TCR^{+} *rag2*$^{-/-}$ (n = 5) and DO11.10 TCR^{+} (n = 9) mice were quantified.

the interaction of coronins with the Arp2/3 complex (*4*, *18*) prompted us to examine the effects of *coronin 1* deficiency on actin polymerization and apoptosis. Fluorescence microscopy and fluorescence-activated cell sorting (FACS) analysis revealed enhanced basal F-actin and, conversely, decreased G-actin in *coronin* $1^{-/-}$ T cells (Fig. 4, A and B); these effects were reversed by reexpression of wild-type coronin 1 (fig. S6S). Additionally, *coronin* $1^{-/-}$ naïve T cells demonstrated a loss in MMP (Fig. 4C). Lastly, incubation with the actin depolymerizing agent latrunculin A partially reversed the loss in MMP and the increased apoptosis observed in *coronin* $1^{-/-}$ T cells (Fig. 4, D and E). Conversely, consistent with studies in T cell lines (*19*), primary T cells treated with jasplakinolide, which decreases actin turnover, demonstrated increased MMP and increased spontaneous apoptosis (Fig. 4, D and E). Thus, actin dynamics in mammalian cells also controls cellular viability through a mitochondrial-dependent pathway.

To determine whether the interaction between coronin 1 and the Arp2/3 complex was required for controlling actin dynamics in T cells, we used a set of coronin 1 mutants, S2D and ΔCC, that were compromised in their ability to bind Arp2/3 (Fig. 4F) (*18*). Whereas expression of wild-type or Flag-tagged wild-type coronin 1 in *coronin* $1^{-/-}$ T cells resulted in decreased phalloidin staining to levels nearing those of *coronin* $1^{+/+}$ T cells, expression of coronin 1 mutants (S2D and ΔCC) did not reverse the enhanced phalloidin staining in *coronin* $1^{-/-}$ T cells (Fig. 4G and fig. S7). Thus, coronin 1 plays an inhibitory role in the steady-state F-actin equilibrium via an Arp2/3-dependent mechanism.

Consistent with an actin-regulatory role of coronin proteins, we have found a selective requirement for coronin 1 in chemokine-induced but not TCR-mediated functions. Our data also indicate an Arp2/3-dependent inhibitory function of coronin 1 on steady-state F-actin equilibrium. Two lines of in vitro experimentation provide the mechanistic basis for this disturbance in actin dynamics. Purified yeast coronin inhibits Arp2/3-mediated actin polymerization (*4*), and electron microscopy (EM) images indicate that coronin binds near p35 (ARPC2) to skew Arp2/3 to a more open and "inactive" conformation (*20*). In *coronin* $1^{-/-}$ T cells, increased F-actin was associated with enhanced apoptosis and shortened survival that was partially reversed with the addition of actin depolarizing agents. This link between actin dynamics and cellular homeostasis may account for the decreased circulating T cell numbers observed in *dock2*$^{-/-}$ and *wasp*$^{-/-}$ mice and in WAS patients with dysregulated cytoskeletal rearrangements (*21*–*23*).

A link between actin dynamics and cellular longevity has been demonstrated recently in yeast (*17*). Yeast with reduced actin dynamics exhibit accumulation of F-actin, loss of MMP, release of reactive oxygen species (ROS), and increased cell death. Conversely, yeast with increased actin dynamics exhibit decreased ROS, increased cellular viability, and prolonged yeast life span. In contrast to our observations on mammalian coronin 1, coronin-null mutants (*crn1*Δ) in yeast have no discernible phenotype. However, *crn1*Δ *act1-159* and *crn1*Δ *cof1-22* mutations accumulate a large filamentous actin mass and exhibit reduced cell growth (*11*). Together, these data indicate that the link between actin dynamics and cellular homeostasis exists in both yeast and mammalian cells. Perturbations in basal F-actin content and decrements in naïve T lymphocyte homeostasis have also been reported in aged mice and humans (*24*, *25*). Our studies reinforce and expand our appreciation of the diversity of functions controlled by the actin cytoskeleton that range from the well-recognized functions of lymphocyte chemotaxis and function to also include lymphocyte survival.

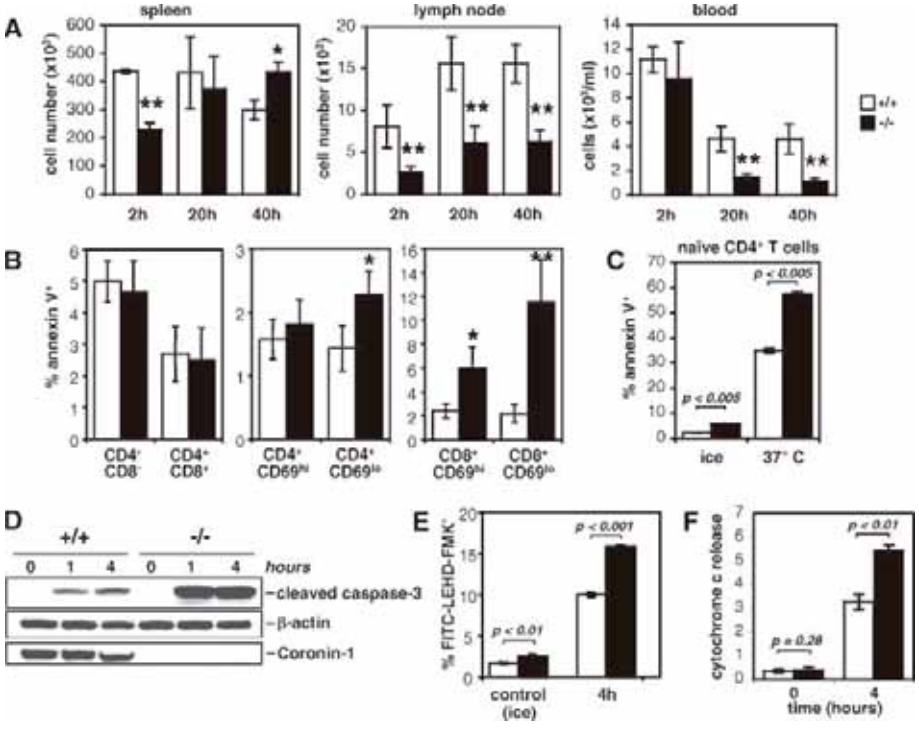

Fig. 3. Survival defect of *coronin* $1^{-/-}$ naïve T cells. Error bars indicate SEM. (**A**) Equal numbers of differentially labeled CD4$^+$ *coronin* $1^{+/+}$ (open bars) and *coronin* $1^{-/-}$ (solid bars) thymocytes were transferred into wild-type mice. Cellular constituents were analyzed at the indicated times by FACS ($n = 4$). (**B**) Percentage of annexin V$^+$ thymocytes from *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ mice ($n = 5$). (**C**) Naïve CD4$^+$ CD62L^{hi} T cells from *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ mice were cultured at 37°C in normal medium or kept on ice for 18 hours. Annexin V$^+$ cells were quantified by FACS staining using duplicate cultures. (**D**) Naïve *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ T cells were cultured in normal medium for the indicated times and then lysed, and cell extracts were analyzed by immunoblotting with mAbs against cleaved caspase-3, β-actin, or coronin 1. (**E**) DO11.10 TCR$^+$ *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ cells were cultured at 37°C, and activation of caspase-9 was assessed by FACS staining. The % of cells with active caspase-9 [(fluorescein isothiocyanate) FITC-LEHD-FMK$^+$] from triplicate cultures was quantified. (**F**) Purified naïve *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ T cells were cultured at 37°C, and postmitochondrial cytosolic fractions were analyzed by immunoblotting with cytochrome c, β-actin, and coronin 1 mAbs (fig. S5A). Densitometric analysis from two independent experiments shows cytochrome c release in arbitrary units after normalization against β-actin.

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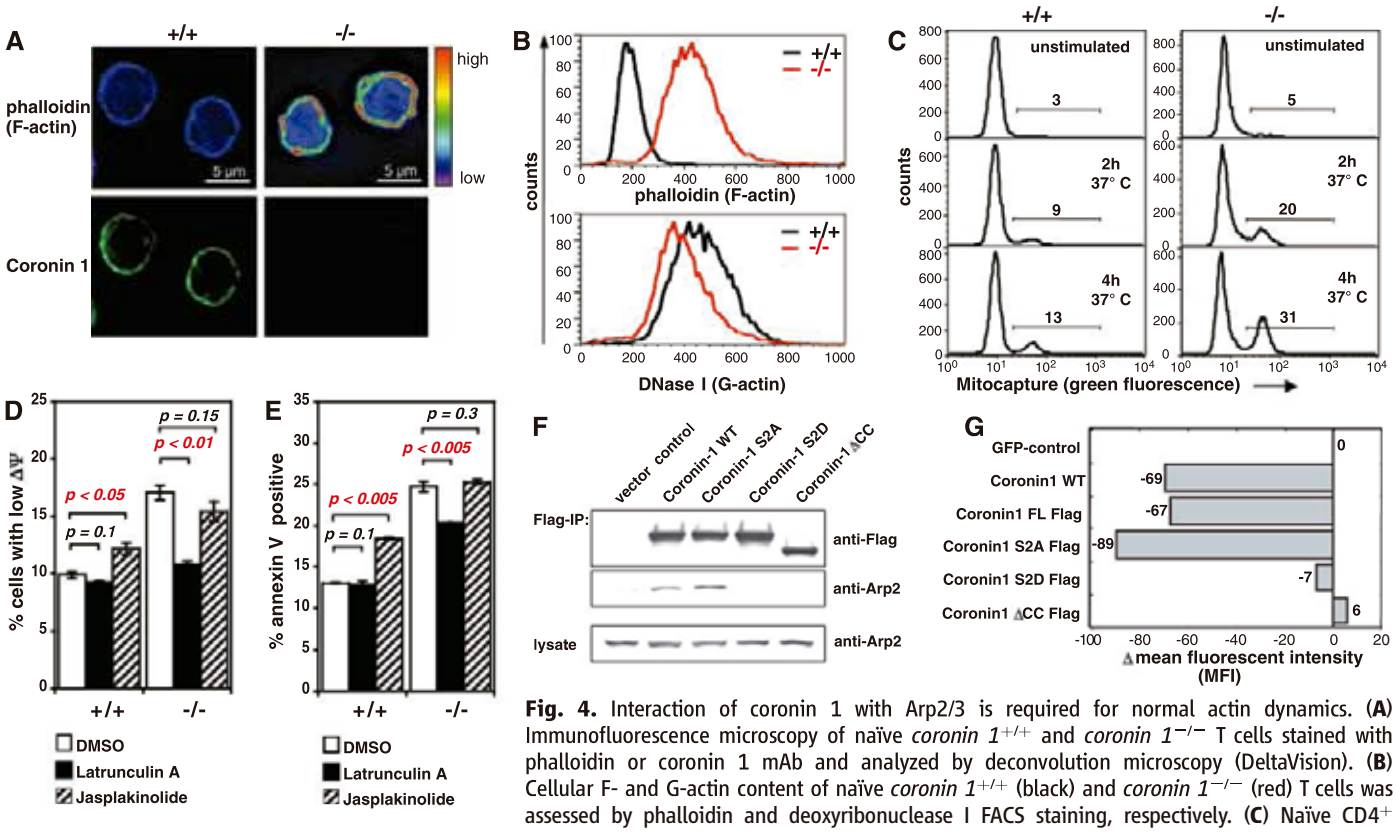

Fig. 4. Interaction of coronin 1 with Arp2/3 is required for normal actin dynamics. (**A**) Immunofluorescence microscopy of naïve *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ T cells stained with phalloidin or coronin 1 mAb and analyzed by deconvolution microscopy (DeltaVision). (**B**) Cellular F- and G-actin content of naïve *coronin* $1^{+/+}$ (black) and *coronin* $1^{-/-}$ (red) T cells was assessed by phalloidin and deoxyribonuclease I FACS staining, respectively. (**C**) Naïve CD4$^+$ DO11.10 TCR$^+$ *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ T cells were cultured at 37°C in normal medium for the indicated times, and changes in the MMP were assessed. (**D** and **E**) Naïve *coronin* $1^{+/+}$ and *coronin* $1^{-/-}$ T cells were preincubated on ice with 5 μg/ml latrunculin A, 1 μM jasplakinolide, or dimethyl sulfoxide (DMSO) carrier. Cells were cultured at 37°C for 2 hours, and loss of MMP (D) or annexin V$^+$ (E) was quantified by FACS. Error bars indicate SD from duplicate cultures. (**F**) Wild-type (WT) and mutant coronin 1 were expressed in A20 cells. Lysates were immunoprecipitated with Flag mAbs and immunoblotted with antibodies against Arp2 and Flag. (**G**) Basal F-actin of DO11.10 TCR$^+$ *coronin* $1^{-/-}$ cell lines, transfected with the indicated coronin 1 internal ribosomal entry site (IRES)–green fluorescent protein (GFP) expression constructs, was assessed by staining with phalloidin and quantified by FACS. Results represent the change in mean fluorescent intensity (ΔMFI) between GFP$^-$ and GFP$^+$ cells. Protein expression was assessed in fig. S7. The ΔMFI between *coronin* $1^{-/-}$ and *coronin* $1^{+/+}$ T cells was –122. Data are representative of two independent experiments.

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Dodeca-CLE Peptides as Suppressors of Plant Stem Cell Differentiation

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In plants and animals, small peptide ligands that signal in cell-cell communication have been suggested to be a crucial component of development. A bioassay of single-cell transdifferentation demonstrates that a dodecapeptide with two hydroxyproline residues is the functional product of genes from the *CLE* family, which includes *CLAVATA3* in *Arabidopsis*. The dodecapeptide suppresses xylem cell development at a concentration of 10^{-11} M and promotes cell division. An application, corresponding to all 26 *Arabidopsis* CLE protein family members, of synthetic dodecapeptides reveals two counteracting signaling pathways involved in stem cell fate.

Organized tissue formation in multicellular organisms requires cell-cell communication. In animals, this process is mediated by many specific extracellular molecules, including peptides. Plant peptides that direct cell-cell communication have not previously been identified, rather a limited number of nonspecific mobile phytohormones such as auxins, cytokinins, and gibberellins direct plant tissue formation (*1*). The recent discovery of peptides in plants, as well as putative leucine-rich repeat (LRR) receptor kinases, suggests that the importance of peptide ligands in plant development may have been overlooked (*2*–*5*). One such signaling system involves both CLAVATA3 (CLV3), a putative peptide ligand, and CLAVATA1 (CLV1), a LRR receptor–like kinase, which determine cell fate in the shoot apical meristem (SAM) (*6*–*8*). LRR-containing receptors also function in cell-cell signaling in animals, although animal receptors do not contain a cytoplasmic kinase domain (*2*).

CLV3 belongs to a large gene family, *CLE* (for *CLV3/ESR*-related), that encodes small proteins with conserved carboxyl termini (*3*, *9*). Genetic and physiological analyses of transgenic plants overexpressing the *CLE* genes (*10*–*13*) and studies using synthetic peptides (*14*) have revealed the involvement of various CLE members in stem cell development; however, the un-

derlying mechanisms remain unknown, as no natural CLE peptide has been identified.

To identify signal molecules responsible for cell-cell interactions, we used a xylogenic culture system, in which isolated *Zinnia* (*Zinnia elegans* L.) mesophyll cells transdifferentiate into tracheary elements (the main conductive cells of the xylem), when cultured in medium containing both naphthaleneacetic acid as an auxin and benzyladenine as a cytokinin (*15*). Using this system, we previously identified xylogen, a unique arabinogalactan protein that mediates inductive cell-cell interaction in vascular development (*16*). During the isolation of xylogen, we noticed that tracheary element differentiation was inhibited by extracellular factor(s), which we isolated, characterized, and named tracheary element differentiation inhibitory factor (TDIF). TDIF was found in a 20% methanol fraction taken from the conditioned medium.

Because auxin and cytokinin are differently involved in the program of tracheary element transdifferentiation (*15*, *17*), we prepared the 20% methanol fraction from the conditioned medium of cultures containing different combinations of naphthaleneacetic acid and benzyladenine (Fig. 1A). We bioassayed TDIF activity in the 20% methanol fraction by adding it at the start of culture to the tracheary element differentiation–induced culture containing both naphthaleneacetic acid and benzyladenine (D culture). The 20% methanol fraction prepared from the culture containing only naphthaleneacetic acid (CN culture) exhibited the highest activity (Fig. 1, A and B). The amount of inhibitory activity in the D culture was much less than that in CN culture. When the TDIF fraction from the conditioned medium of CN culture was added at the start of culture, tracheary element differentiation was almost completely inhibited (Fig. 1C), whereas cell division was promoted (Fig. 1D). These opposing biological activities suggest that TDIF specifically suppresses the tracheary element differentiation pathway, but does not affect general physiological activity.

To address which precise processes were suppressed during tracheary element differentiation, we added the TDIF fraction to D culture at different times. The process of tracheary element differentiation in culture is divided into three stages; stage one (0 to 24 hours) in which mesophyll cells dedifferentiate, stage two (24 to 48 hours) in which dedifferentiated cells differentiate into procambium cells as stem cells, and stage three (48 to 96 hours) in which stem cells differentiate into tracheary elements (*15*). The addition of TDIF completely inhibited tracheary element differentiation until 36 hours after culture initiation (fig. S1A). RNA gel-blot analyses with stage-specific marker genes (*18*) suggested that TDIF preferentially suppressed the entry into stage 3; the transition of procambial cells to tracheary elements (Fig. 1E).

To characterize the chemical properties of TDIF, we treated the biologically active fraction with pronase E, trypsin, proteinase K, and denatured proteases (fig. S1B). Proteinase K did not affect TDIF activity; pronase E, but not its denatured form, eliminated most activity; and trypsin slightly reduced activity. These results indicate that TDIF is a proteinaceous factor. TDIF was hypothesized to be a small peptide because it passed through a 5-kD cutoff membrane (Biomax-5, Millipore) during ultrafiltration experiments (fig. S1C) and was not inactivated when exposed to 100°C for 10 min (fig. S1D).

We fractionated TDIF by high-performance liquid chromatography in association with a bioassay (Fig. 2, A to C) and finally isolated a peptide that appeared as a single peak (Fig. 2C). The combination of tandem mass spectrometry analysis and amino acid sequencing revealed that TDIF is a dodecapeptide with two hydroxyproline residues (Hyp), HEVHypSGHypNPISN (fig. S2) (*19*, *20*). Synthetic TDIF at 30 pM, corresponding to the concentration in the CN medium, inhibited tracheary element differentiation by 50% (Fig. 3A). The full-length cDNA corresponding to TDIF was isolated from *Zinnia* cells cultured in CN medium (Fig. 2D). The cDNA potentially encodes a

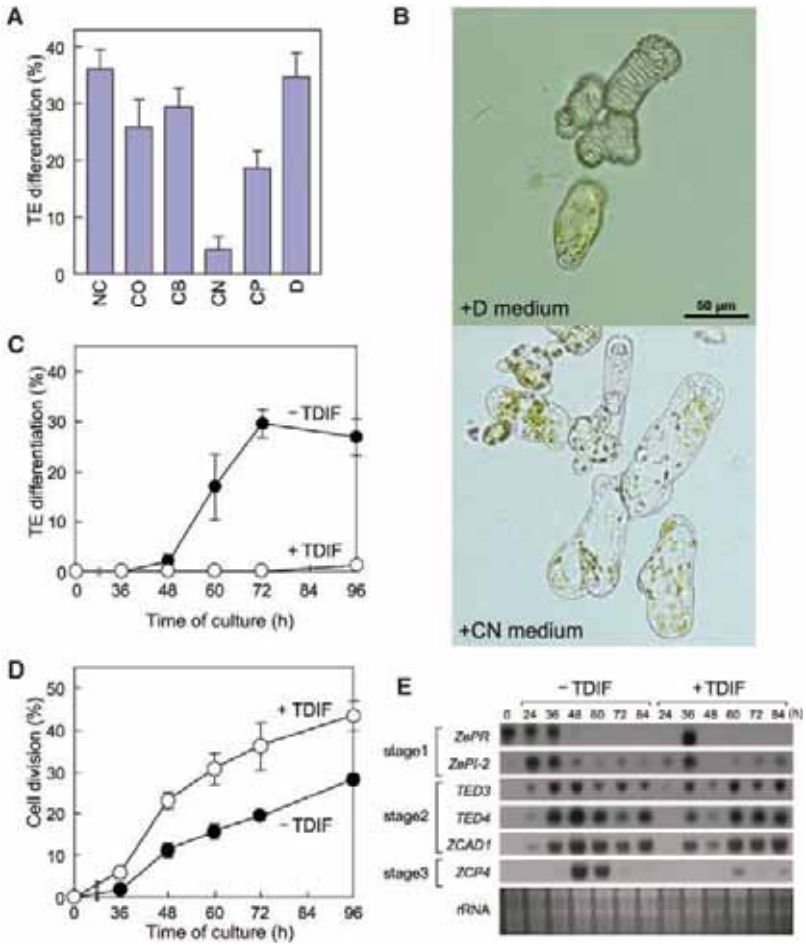

Fig. 1. TDIF activity in cultured medium. (**A**) TDIF activity varies among different cultures (CO, hormone free; CB, only benzyladenine (BA); CN, only naphthaleneacetic acid (NAA); CP, low BA and NAA; D, both BA and NAA). *Zinnia* mesophyll cells were cultured in CO, CB, CN, CP, and D media for 72 hours. A 20% methanol fraction of each medium was added to D culture at the start of culture, at a final concentration equivalent to threefold the original concentration. NC, no addition. (**B**) Tracheary element differentiation is inhibited by the 20% fraction of CN culture medium, but not D culture medium. (**C**) TDIF inhibits tracheary element differentiation. (**D**) TDIF increases cell division over time. (**E**) TDIF suppresses mRNA accumulation of a stage three–specific gene, but not those specific to stages one or two. The progress of tracheary element differentiation was monitored by the expression of stage-specific marker genes, *ZePR* (*Zinnia elegans* pathogenesis-related gene, AB091075), *ZePI-2* (gene for *Zinnia elegans* protease inhibitor-2, AB091074), *TED3* (tracheary element differentiation–related gene 3, D30801), *TED4* (tracheary element differentiation–related gene 4, D30802), *ZCAD1* (*Zinnia* cinnamyl alcohol dehydrogenase gene, D86590), and *ZCP4* (*Zinnia* cysteine protease gene 4; AB091070), as described (*18*).

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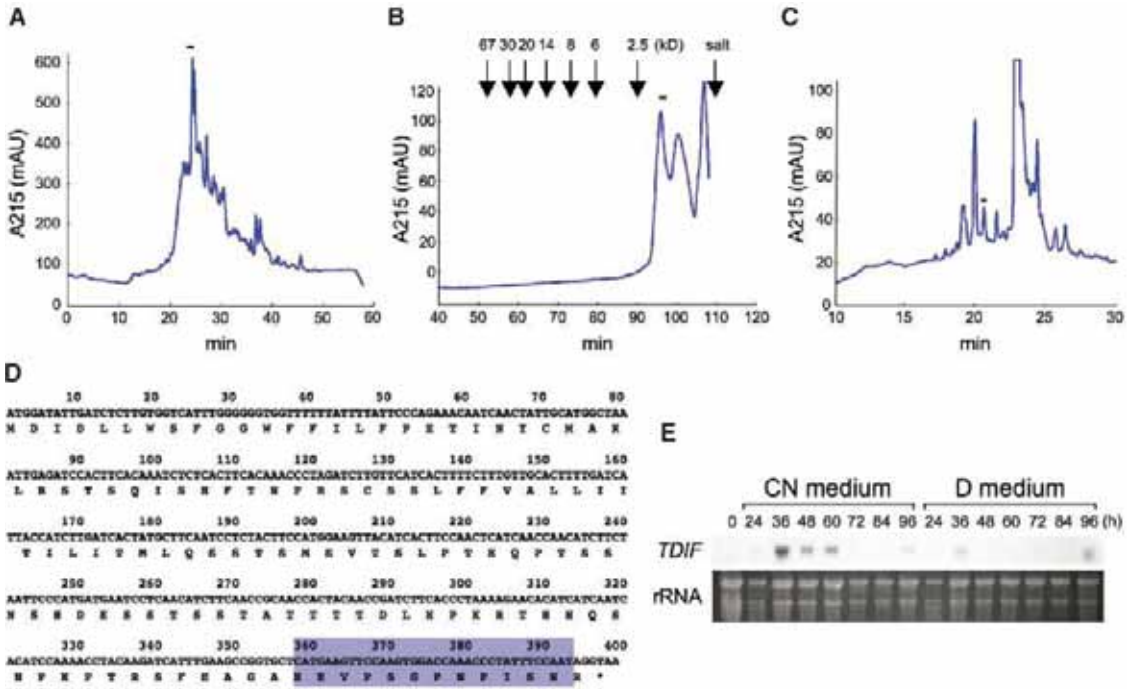

Fig. 2. Identification of TDIF. (**A**) Poly(2-sulfoethyl aspartamide) ion-exchange column chromatography. The horizontal bar indicates an active fraction. (**B**) Gel-filtration column chromatography profile using tandem Super SW 3000 columns. (**C**) An inertsil ODS3 reversed-phase column chromatography profile. (**D**) The deduced amino acid sequence of a TDIF gene from *Zinnia elegans*. (**E**) Time course of TDIF mRNA accumulation in cells cultured in CN and D medium.

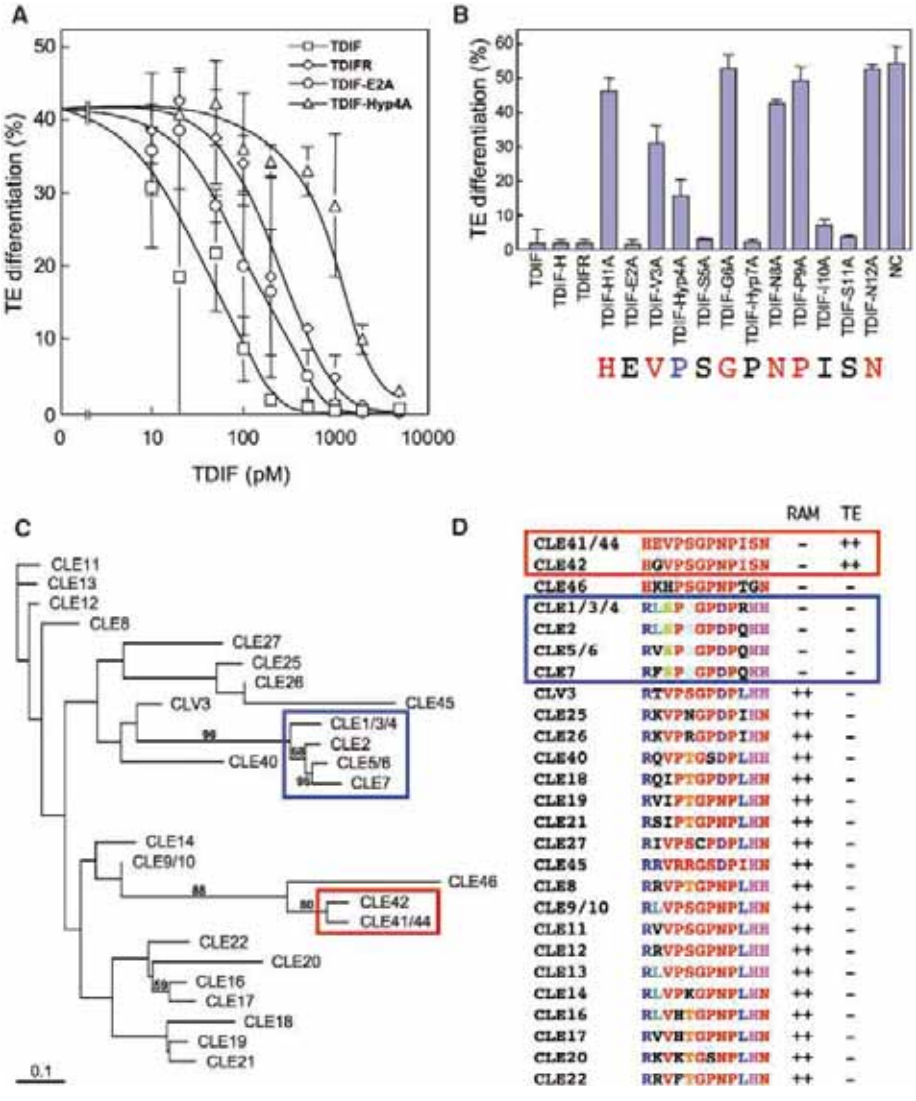

Fig. 3. Functional analysis of dodeca-CLE peptides. (**A**) Kinetics of the inhibition of tracheary element differentiation by TDIF and TDIF derivatives. (**B**) The inhibition of tracheary element differentiation by TDIF derivatives at 8 nM. Each amino acid residue of TDIF was replaced by alanine (TDIF-H1A to TDIF-N12A). TDIF-R had an additional arginine added at the C terminus. In TDIF-H, the two Hyp residues were replaced by proline. NC, no addition. (**C**) An unrooted neighbor-joining tree of *Arabidopsis* dodeca-CLE peptides (table S1). Bootstrap values of 50% and above from the neighbor-joining method, with Kimura's correction (*25*), are shown. The scale bar indicates the number of amino acid substitutions per site. (**D**) Amino acid composition and function of the 26 synthetic dodeca-CLE peptides with two hydroxyproline residues. The suppression of tracheary element differentiation (TE) and root apical meristem (RAM) formation (*14*) by the dodeca-CLE peptides was examined.

protein of 132 amino acids, but only 12 from H^{120} to N^{131} match the TDIF sequence. This result indicates that TDIF is produced through the removal of residues M^1 to A^{119} at the N terminus and R^{132} at the C terminus. The level of transcripts for the TDIF precursor was high between 36 and 60 hours in CN-cultured cells and present at lower levels in D-cultured cells after 36 and 96 hours (Fig. 2E). This result is consistent with the appearance of the TDIF activity in CN culture (fig. S1E) and the weak TDIF activity in D culture (Fig. 1A).

Through homology searches, the TDIF sequence was found to be the same as the *Arabidopsis* C-terminal 12 amino acids CLE41 and CLE44, and highly homologous to CLE42 and CLE46 (fig. S3). Although many studies have suggested that the *CLE* gene family produces functional small peptides (*3*, *9*–*14*), there has been no direct evidence of CLE peptides in situ. The identification of TDIF is direct evidence that a 12–amino acid peptide functions as an extracellular signaling molecule in plants.

To deduce the chemical basis of CLE function, we made TDIF derivatives and compared their relative activities (Fig. 3, A and B). Hydroxylation of the two proline residues was not necessary for TDIF activity; however, addition of an arginine at the C terminus, which is the precursor sequence, reduced activity by 1/7th compared with the wild-type peptide. Alanine scanning experiments indicated that replacement of the amino acids at positions 2, 5, 7, 10, and 11 within the peptide did not affect TDIF activity (Fig. 3B). In contrast, substitution of alanine for H^1, V^3, G^6, N^8, P^9, and N^{12} all caused a severe loss of TDIF activity. A substitution of the 4th amino acid reduced activity by 1/30th (Fig. 3A).

The in situ CLV3 peptide is 12 amino acids long and contains two hydroxyproline residues (*21*). Tracheary element differentiation and root growth were investigated with bioassays of 26 synthetic peptides of 12 amino acids, including the two hydroxyprolines, corresponding to the predicted products of all of the 31 *Arabidopsis CLE* genes (Fig. 3, C and D, and table S1). Dodecapeptides from CLE42 and CLE41/CLE44 have strong TDIF activity (Fig. 3D), but did not inhibit root growth. On the other hand, the dodecapeptides of all other CLE proteins lacked TDIF activity. Therefore, CLE42 and CLE41/CLE44 may play a specific role in xylem differentiation.

Like CLE42 and CLE41/CLE44, five peptides (CLE46, CLE1/CLE3/CLE4, CLE2, CLE5/CLE6, and CLE7) did not suppress root growth, whereas the others did so strongly (Fig. 3D). In accordance with overexpression of the *CLV3* (*8*) and various *CLE* genes except the *CLE1* to *CLE7* genes (*10*–*14*), our results demonstrate that most dodeca-CLE peptides suppress root growth. Nevertheless, the clade composed of CLE42 and CLE41/CLE44 displayed distinctive functions; the clade composed of CLE1/CLE3/CLE4, CLE2, CLE5/CLE6, and CLE7 did not exhibit any detectable activity in our bioassay, although the CLE1 to CLE7 peptides have been reported to function in the shoot apical meristem (*12*, *13*).

Alanine scanning mutagenesis, along with comparison of the amino acid sequence among CLE peptides, has identified the amino acids necessary for both general and specific activity of CLE peptides. G^6 and P^9, which are absolutely conserved in almost all *Arabidopsis* CLE peptides, and the slightly less conserved residues, V^3, N^8, and N^{12}, were essential for TDIF activity. Therefore, these amino acids may be required for the general activity of CLE peptides. H^1 may confer distinct biological activity as it is unique among CLE peptides with TDIF activity. Our data, along with in situ identification of the CLV3 peptide (*21*), indicate that the active form of CLE proteins is a dodecapeptide containing two hydroxyprolines.

Previous analysis indicates that the major function of CLV3, which is a putative ligand to the CLV1 or CLV1-CLV2 receptor complex, is the suppression of stem cell proliferation in the meristem (*2*, *22*, *23*). Excess CLV3 seems to promote differentiation from stem cells (*8*, *14*). In contrast, TDIF suppresses the differentiation of xylem cells from stem cell–like procambial cells and promotes cell division. Indeed, a functional CLV3 peptide promotes xylem cell differentiation in *Zinnia* cell culture (fig. S4). Therefore, there are two counteracting pathways in CLE signaling, one that promotes and one that inhibits stem cell differentiation in vascular development. It will be interesting if two similar counteracting pathways function in meristems.

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19. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
20. Hydroxylation of proline is a frequent modification of secretory proteins and peptides in plants. We have reported the motifs necessary for proline hydroxylation and hydroxyproline glycosylation in plant secretory proteins (*24*). The hydroxylation, but not glycosylation, of the two prolines of TDIF conforms well to this rule. Although hydroxylation of prolines is not necessary for activity of TDIF, the hydroxylation may render the peptide more hydrophilic, facilitating its movement and its resistance against proteolysis in apoplastic space.
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Supporting Online Material

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A Plant Peptide Encoded by *CLV3* Identified by in Situ MALDI-TOF MS Analysis

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The *Arabidopsis CLAVATA3* (*CLV3*) gene encodes a stem cell–specific protein presumed to be a precursor of a secreted peptide hormone. Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) applied to in situ *Arabidopsis* tissues determined the structure of a modified 12–amino acid peptide (MCLV3), which was derived from a conserved motif in the CLV3 sequence. Synthetic MCLV3 induced shoot and root meristem consumption as cells differentiated into other organs, displaying the typical phenotype of transgenic plants overexpressing *CLV3*. These results suggest that the functional peptide of CLV3 is MCLV3.

Several lines of evidence indicate that peptides are important for plant growth and development. Three plant peptide hormones, systemins, phytosulfokine, and SCR/SP11, have been biochemically identified. These bioactive peptides are originated and posttrans-

lationally modified from corresponding precursor proteins that possess signal sequences in each N-terminal region (*1*). More than 200 leucine-rich repeat (LRR) type receptor-like kinases (RLKs) have been identified in the *Arabidopsis* genome, and these are presumed to act as receptors for peptides or small molecules. The mutants of these receptors show interesting phenotypes in plant growth and development (*2*). From the presence of these putative receptors, we hypothesize that putative peptides exist that interact with these receptors, indicating that many unidentified hormones may play important roles in plant growth and development. However, the mature forms of predicted peptide hormones are unknown, and we cannot identify the mature forms via "in silico" (computer analysis) approaches.

Stem cells in higher plants proliferate and supply the new cells destined to become various organs while also maintaining undifferentiated cells in the apical meristem. The *CLAVATA* genes (*CLV1*, *2*, and *3*) control the size of the shoot apical meristem (SAM) in *Arabidopsis* (*3*, *4*). Loss-of-function mutants of *CLAVATA* genes accumulate stem cells in the SAM, resulting in an enlarged, dome-shaped meristem. The *CLV1* gene encodes an LRR-RLK, whereas *CLV2* encodes a similar protein lacking the kinase domain (*5*, *6*). *CLV1* is expressed only in the central L3 layer of the SAM, and the resulting protein is hypothesized to form a heteromeric receptor complex with CLV2. *CLV3* is expressed at the outer L1 and middle L2 layer, adjacent to the zone expressing *CLV1*, and is hypothesized to encode a ligand of the CLV1/CLV2 receptor complex. The *CLV3* gene encodes a 96–amino acid protein with an 18–amino acid N-terminal signal peptide (*7*). *CLV3* belongs to the *CLE* gene family (*CLAVATA3/ESR-related*), which shares a conserved, 14–amino acid CLE motif at the C-terminal region (*8*).

We generated transgenic plants constitutively overexpressing *CLV3* under the control of the cauliflower mosaic virus *35S* promoter (*CaMV35S*). *CaMV35S::CLV3* transgenic plants (*CLV3OX*) ceased initiating organs from the SAM after the emergence of the first leaves, which were occasionally misshapen (*9*). Four lines of plants exhibiting premature cessation of leaf development were selected, and dissected leaf tissues were used to induce calli. During dedifferentiation and callus formation, we observed no differences between wild-type and *CLV3OX* plants (fig. S1A). We maintained dedifferentiated calli, and *CLV3* mRNA overexpression in the *CLV3OX* calli was confirmed by Northern blotting (fig. S1B).

Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) was used to identify the neuropeptides involving neural signal transduction in crustaceans (*10*). We characterized the structure of the mature peptide encoded by the *CLV3* gene (MCLV3) in *CLV3OX* calli using MALDI-TOF MS. We performed in situ MALDI-TOF MS experiments with *CLV3OX* calli slices to detect typical ions (fig. S2). We observed a *CLV3OX*-specific ion at a mass-to-charge ratio (*m*/*z*) of 1345.6 (Fig. 1A) when calli were cultured on hormone-free agar medium for 6 days. The observed ion was due to a peptide containing 12 amino acid residues from Arg^{70} to His^{81} in CLV3, in which two of three proline residues were modified to hydroxyproline (P^h or Hyp). This was the only peptide specifically detected in *CLV3OX* calli and, therefore, we hypothesize that this peptide is the only CLV3-derived peptide present in the tissue. Thus, MCLV3 is the same peptide as the identified peptide.

We subjected the pseudomolecular ion (*m*/*z* = 1345.6) to MS/MS analysis to identify which of the three prolines were hydroxylated. We identified one strong (at *m*/*z* = 842.4) and four weak ion peaks (at *m*/*z* = 503.3, 825.4, 1189.6, and 1207.6) (Fig. 1B). We determined that the *m*/*z* = 842.4 ion was derived from the N-terminal $RTVP^hSGP^hD$ fragment and that the *m*/*z* = 503.3 ion was derived from the C-terminal PLHH fragment (Fig. 1C). We hypothesize that the C-terminal end contains a His residue, because the observed fragment ion (*m*/*z* = 1189.6) originated from the loss of a C-terminal His residue accompanied by a hydrated ion (*m*/*z* = 1207.6).

To confirm the structure of MCLV3, we synthesized three dodecapeptides containing two Hyp residues covering all possible combinations. The MS and MS/MS spectra of one synthetic peptide agreed with that of the natural peptide, confirming its structure (Fig. 1D). The MS/MS analysis of the two peptides that contained Hyp residues at incorrect positions resulted in different-fragment ions from those of MCLV3 (fig. S3), leading us to conclude that $RTVP^hSGP^hDPLHH$ is the structure of the MCLV3 peptide.

We examined the biological activities of MCLV3 by observing the effect of synthesized CLV3 peptides of various lengths (Fig. 2A) on roots on agar plates. Wild-type seeds were exposed to 1 μM MCLV3 and control buffer, resulting in main root lengths at 14 days after germination of 18.6 ± 2.0 mm (mean ± SEM) and 85.0 ± 6.3 mm, respectively (Fig. 2, B and C); these results show that MCLV3 limits root growth. Treatment with the 14–amino acid CLV3, CLE19, and CLE40 peptides also result in a shortened root (*11*). The Arg^{70} (R^{70}) position in CLV3 is hypothesized to be excluded from the functional CLV3 peptide (*12*). After exposure to CLV3L and CLV3S (1 μM), the lengths of the main root were 18.4 ± 2.4 and 78.2 ± 10.5 mm, respectively (Fig. 2B). These results indicate that the functional CLV3 peptide contains the R^{70} residue at the N terminus, which is the second R of the CLE motif, consistent with the results of the in situ MALDI-TOF MS analysis.

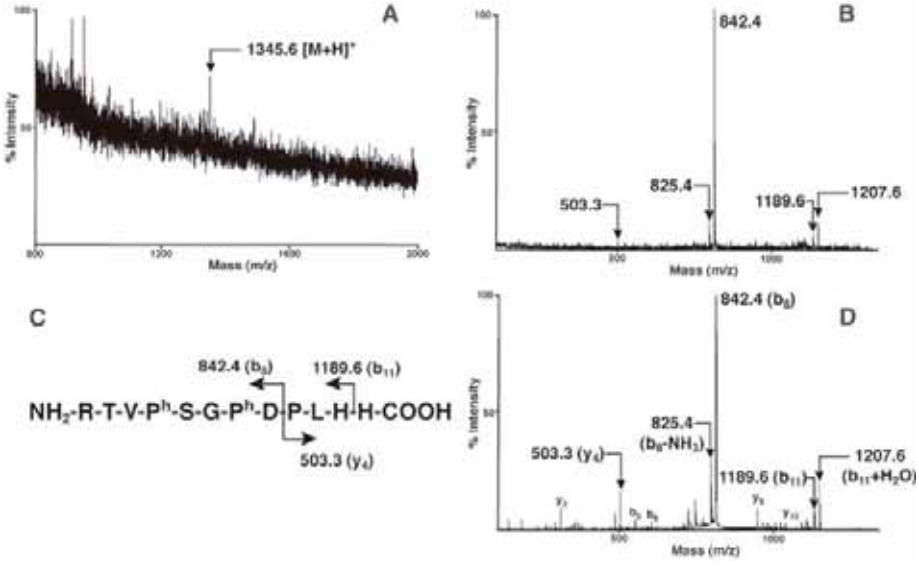

Fig. 1. In situ MALDI-TOF MS analysis of *CLV3OX* callus. (**A**) A typical ion (*m*/*z* = 1345.6) observed from a *CLV3OX* callus. (**B**) MS/MS spectrum of fragment ions from MCLV3. One major (*m*/*z* = 842.4) and four minor (*m*/*z* = 503.3, 825.4, 1189.6, 1207.6) ions were detected. (**C**) The predicted structure of MCLV3; "b-series" or "y-series" fragment ions originating from N- and C-terminal fragments from peptide bond cleavage between -CO- and -NH- are identified. Subscript numbers indicate the length of each fragment. (**D**) MS/MS spectrum of synthetic MCLV3. Five signals assigned to b_8, b_8-NH_3, b_{11}, $b_{11} + H_2O$, and y_4 fragments correspond with those in spectrum B. Several ions are assigned to fragment ions (b_5, b_6, y_2, y_9, y_{10}) originating from synthetic MCLV3.

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To delimit the minimal C-terminal region required for activity, and to examine the effect of the C-terminal region outside of the CLE motif, we examined CLV3L, -A, -B, and -C (Fig. 2). The root meristem was inhibited by 1 μM applications of CLV3L, CLV3-A, and CLV3-B. In contrast, applications of the CLV3-C peptide did not affect the root meristem (Fig. 2C), indicating that the 13th His in the CLE motif is essential for activity. Therefore, the C-terminal region outside the CLE motif is superfluous for activity and MCLV3, the 12–amino acid CLV3 peptide, spans the 2nd "R" to the 13th "H" in the CLE motif. As identified by the in situ MALDI-TOF MS, this molecule was the most active and shortest functional CLV3 peptide. Hydroxylation of the two proline residues did not affect root growth inhibition when exposed to MCLV3 compared to MCLV3′, and CLV3-C compared to CLV3-C′ (Fig. 2C).

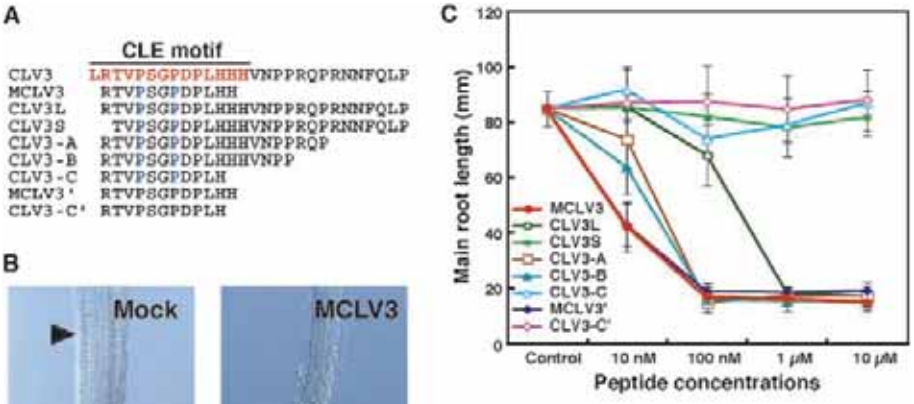

Fig. 2. MCLV3 and related peptides function in the root apical meristem. (**A**) The amino acid sequences of CLV3 (residues 69 to 96) and MCLV3-related peptides. Blue, hydroxylated proline residues; red, amino acids of CLE motif. (**B**) The effect of MCLV3 and related peptides on root meristem. Wild-type plants were grown for 14 days on agar plates containing buffer (Mock) or 1 μM each of MCLV3, CLV3L, or CLV3S, and primary root morphology was observed. Scale bar: 100 μm. Arrowheads indicate border between root meristematic and elongation zones. (**C**) Effect of various peptide concentrations on wild-type root development. Main root length was measured after 14 days of growth on media containing peptide (n = 10 for each peptide). Abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; and V, Val.

To examine the effect of synthetic CLV3 peptides on the SAM, we cultured seedlings in liquid medium and exposed them to 1 μM applications of different synthetic peptides. Applications of CLV3S and CLV3-C peptides had no effect on the SAM (Fig. 3, A to C). In contrast, CLV3L, CLV3-A, CLV3-B, and MCLV3 reduced the size of the SAM (Fig. 3, D to G). Therefore, the effects of various CLV3 peptides on the SAM resembled those on root growth.

Scanning electron microscopy revealed that MCLV3 (Fig. 3K) and CLV3L (Fig. 3J), as well as CLV3-A, CLV3-B, and MCLV3′ (fig. S4), induced random phyllotaxis and the occasional severe reduction of the SAM region compared to the control (Fig. 3I). These phenotypes are similar to those of *CLV3OX* or *wuschel* seedlings (*9*, *13*). On the contrary, phyllotaxis was normal in seedlings exposed to CLV3S and CLV3-C peptides (fig. S4). In about 5% of plants treated with these functional CLV3 peptides, a leaflike structure was produced at the top of the SAM (Fig. 3, H and L), but these leaflike structures maintained phenotypically normal abaxial-adaxial polarity.

Thus, MCLV3 is the minimal unit that mimics *CLV3* signaling at the SAM. MCLV3 was composed of 12 amino acid residues containing two Hyp residues, the size and the position of which were identical to those of the CLE peptide, TDIF (tracheary element differentiation inhibitory factor), which was isolated from *Zinnia* as an inhibitory factor of xylem cell differentiation (*14*). We hypothesize that the chemical structure of MCLV3 should be common to many CLE peptides. The fact that the activity of the longer peptide, CLV3L, was weaker than that of MCLV3 indicates that processing into 12–amino acid peptides is crucial to the construction of an active and mature CLV3 peptide.

The 4-hydroxylation of proline residues is a posttranslational modification found in animal tissues (*15*). In higher plants, this modification occurs in many glycoproteins, including arabinogalactan proteins and elastins (*16*), as well as in the bioactive peptide systemins in tobacco and tomato (*17*). Hydroxylation had no effect on MCLV3 activity. Therefore, the Hyp residues may not be responsible for the affinity with which MCLV3 binds to its receptor. Hydroxylation may serve other functions such as trafficking, storage, and peptide stability.

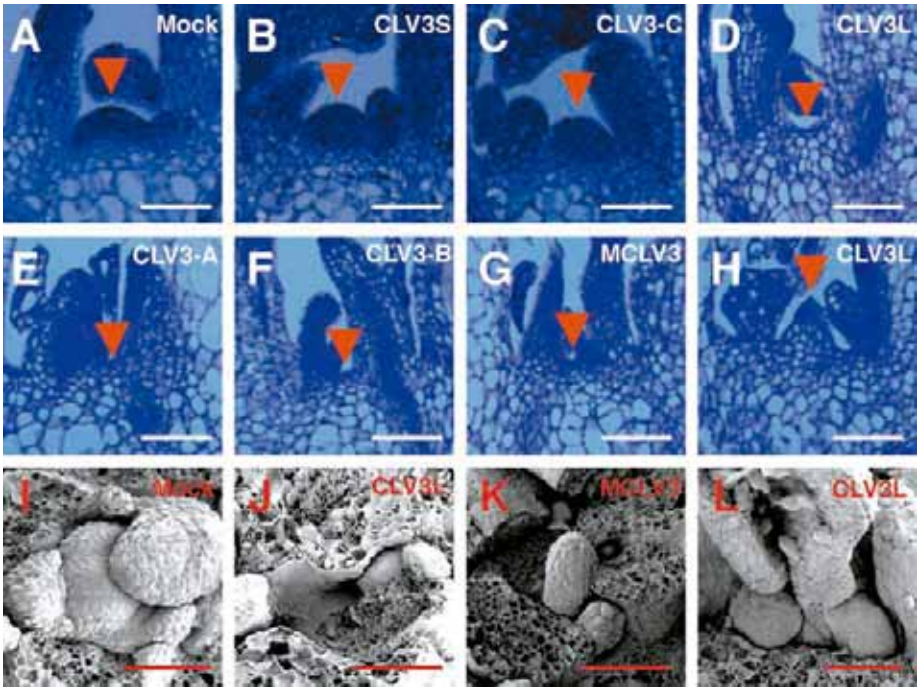

Fig. 3. MCLV3 and related peptide functions in SAM. Longitudinal sections (**A** to **H**) and scanning electron micrographs (**I** to **L**) of wild-type plants (Col-0) incubated with 1-μM peptides in liquid Murashige-Skoog medium for 21 days after germination. Scale bar: 100 μm.

The 216 LRR-RLKs represent the largest group of RLKs in *Arabidopsis* (*18*). It has long been known that CLV3-CLV1 interactions are critical for the proper development of the SAM but, because the active form of CLV3 was unknown, the direct ligand-receptor interaction in the CLAVATA pathway had not been demonstrated. Our identification of the chemical structure of the CLV3 peptide will allow the interaction between CLV3 and its putative receptors, CLV1 and CLV2, to be clarified.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S4
References

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Escherichia coli Induces DNA Double-Strand Breaks in Eukaryotic Cells

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Transient infection of eukaryotic cells with commensal and extraintestinal pathogenic *Escherichia coli* of phylogenetic group B2 blocks mitosis and induces megalocytosis. This trait is linked to a widely spread genomic island that encodes giant modular nonribosomal peptide and polyketide synthases. Contact with *E. coli* expressing this gene cluster causes DNA double-strand breaks and activation of the DNA damage checkpoint pathway, leading to cell cycle arrest and eventually to cell death. Discovery of hybrid peptide-polyketide genotoxins in *E. coli* will change our view on pathogenesis and commensalism and open new biotechnological applications.

Escherichia coli is both the most common cause of infections by Gram-negative bacilli and a commensal of the normal gut microflora (*1*). The versatility of this pathogen arises from production of a diverse array of virulence factors that manipulate basic host cell functions (*2*, *3*), such as the cyclomodulins, which target the host cell cycle and influence whether an infected cell will grow and divide, or die (*4*). We observed that certain *E. coli* strains induce megalocytosis in cultured eukaryotic cells, characterized by a progressive enlargement of the cell body and nucleus and the absence of mitosis (Fig. 1). This cytopathic effect, reminiscent of the effect of cyclomodulins, was observed upon transient infection of different mammalian cells (HeLa, CHO, A375, and IEC-6) and induced by pathogenic *E. coli* strains isolated from meningitis and urinary tract infections and by certain commensal strains but not by laboratory K-12 strains nor by enteropathogenic or enterohemorrhagic *E. coli* (Fig. 1A, fig. S1, and table S1). The cytopathic activity was contact-dependent and was not observed when bacteria were separated from mammalian cells by a 0.2-μm permeable membrane. Inhibition of bacterial internalization with cytochalasin-D did not abrogate the cytopathic effect. Heat-killed bacteria, gentamicin-killed bacteria, bacterial culture supernatants, and bacterial lysates were not cytopathic (fig. S2). This effect could not be explained by the production of toxins known to alter the host cell cycle such as Cytolethal Distending Toxins (CDT) (*5*), Cycle Inhibiting Factor (*6*), or Cytotoxic Necrotizing Factors (*7*), or by the production of α-hemolysin (*8*). Engineered mutants and strains devoid of these toxin genes remained cytopathic (table S1).

To identify the bacterial genes involved in this phenotype, we generated transposon mutants in two cytopathic *E. coli* strains. Negative mutants had transposons clustered in a 54-kilobase chromosomal region (Fig. 2) that exhibited typical features of a genomic island and was inserted in the *asnW* tRNA locus, an integration hotspot for foreign mobile DNA elements (*2*). The genomic island was fully sequenced in newborn meningitis strain IHE3034, and the presence of an identical genomic island was confirmed in newborn meningitis strain SP15 (*9*), commensal strain Nissle 1917 (*10*), and uropathogenic strain CFT073 (*11*). To show the involvement of this genomic island in the induction of the megalocytosis phenotype, we deleted the entire island in IHE3034, resulting in a noncytopathic mutant (fig. S3). In contrast, laboratory *E. coli* strain DH10B hosting a bacterial artificial chromosome (BAC) bearing the complete genomic island triggered megalocytosis and proliferation arrest in transiently infected cells, whereas DH10B harboring the empty BAC vector did not (fig. S3).

To test the distribution of this genomic island within the species *E. coli*, we performed a survey on 55 intestinal pathogenic *E. coli* strains (enteroinvasive, enteropathogenic, enterohemorrhagic, enterotoxigenic, and enteroaggregative *E. coli*), 97 extraintestinal pathogenic *E. coli* (ExPEC) strains, and 32 strains isolated from the feces of healthy individuals. Polymerase chain reaction (PCR) screening indicated that this genomic island is absent in intestinal pathogenic *E. coli* strains, but present in 53 and 34% of the ExPEC and fecal isolates, respectively. Furthermore, PCR screening of the complete *E. coli* reference collection indicated that this genomic island is restricted to, and widely distributed in, the B2 phylogenic group that comprises commensals and ExPEC strains (*12*, *13*) (fig. S4).

The genomic island, hereafter named *pks* island, encodes a machinery for the synthesis of peptide-polyketide hybrid compounds. This machinery consists of three nonribosomal peptide megasynthases (NRPS); three polyketide megasynthases (PKS); two hybrid NRPS/PKS

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megasynthases; and nine accessory, tailoring, and editing enzymes (table S2). NRPS and PKS are large multifunctional enzymes, found in bacteria and fungi, that produce an immense variety of peptides and polyketides of broad structural and biological activity (*14*, *15*). In silico analysis of the megasynthases encoded by the genomic island revealed a typical but complex modular structure (Fig. 2). Noteworthy is the thiazole-forming NRPS module in ClbK (composed of heterocyclization, cysteine-specific adenylation, oxidation, and peptidyl carriage domains). Thiazole rings are signature pharmacophores common to many natural products and are important functional elements [e.g., intercalating DNA as in the case of the peptide-polyketide bleomycin (*16*)].

Systematic mutagenesis of the *pks*-island genes in DH10B harboring the *pks* island on a BAC (BAC*pks*) showed that all of the PKS and NRPS and eight of the nine accessory and tailoring enzymes were required to induce the cytopathic effect (Fig. 2 and table S2). Only mutation of the gene coding for a putative efflux pump (*17*) did not alter the cytopathic activity, possibly because other efflux pumps encoded elsewhere on the chromosome could rescue this mutation. Reverse transcription PCR experiments indicated that the genes of the *pks* island were transcribed under in vitro conditions, as well as during contact with host cells (fig. S5). Together, these genetic and functional analyses indicate that the *E. coli* *pks* island codes for a polyketide-peptide hybrid cytotoxin.

To characterize the mode of action of this new cytotoxin, we examined the cell cycle of infected mammalian cells exposed to cytopathic *E. coli* strains. Flow cytometry analyses showed that the nucleus of the giant cells had a 4n DNA content (Fig. 3A). This observation, together with the absence of dividing cells (Fig. 1B), indicates that giant cells were blocked at the G_2/M transition. Time-course experiments in which cells were synchronized at the G_1/S transition and then exposed to bacteria showed that DH10B BAC*pks*-exposed cells lagged in S phase for 48 hours and eventually accumulated in G_2/M, whereas control cells went through S phase in less than 12 hours and continued a normal cell cycle (Fig. 3A). We examined whether the G_2 checkpoint that stops the cell cycle in response to DNA injury was activated (*18*). Ataxia-telangiectasia mutated protein (ATM), a central protein in DNA damage response (*19*), was activated in DH10B BAC*pks*-exposed cells together with the ATM signal-transducer Chk2 (Fig. 3B). Chk2 is known to phosphorylate Cdc25C protein, resulting in its inactivation by cytoplasmic retention by 14-3-3 proteins. As expected, we observed that Cdc25C was excluded from the nuclei of DH10B BAC*pks*-exposed cells (Fig. 3C). Consistent with the nuclear exclusion of Cdc25C, we observed high levels of inactive phosphorylated (Tyr^{15}) form of Cdk1 in DH10B BAC*pks*-exposed cells (Fig. 3B), thus explaining the G_2/M block. Further evidence that the G_2 checkpoint is activated in cells exposed to cytopathic *E. coli* was obtained by inhibiting ATM with caffeine (*20*). The G_2 block was alleviated, as a substantial number of cells reentered M-phase upon caffeine treatment (Fig. 3D). Hence, the DNA damage signaling cascade, starting with ATM activation, is activated upon exposure to *E. coli* harboring the *pks* island.

To examine whether exposure to *E. coli* harboring the *pks* island inflicts DNA injury to host cells, we monitored the phosphorylation of histone H2AX, a sensitive marker of DNA double-strand breaks (DSBs) (*21*). Both HeLa cells and nontumor intestinal crypt IEC-6 cells exhibited nuclear phosphorylated H2AX (γH2AX) within 4 hours (Fig. 4A and fig. S6). The γH2AX signal of the infected DH10B BAC*pks* cell population increased in a dose-related manner, ranging from distinctive nuclear foci to pan-nuclear response, reaching saturation at an infectious dose of 100 bacteria per cell (Fig. 4, A and B). Twenty-four hours after infection with low dose of DH10B BAC*pks* (20 bacteria per cell), a subset of cells showed background levels of γH2AX (Fig. 4B), suggesting that these cells endured moderate DNA damage and repaired their DNA. The occurrence of DSBs in infected cells was confirmed with the use of the single-cell gel electrophoresis (comet) assay. Four hours after exposure to bacteria, DNA lesions were detected in cells exposed to DH10B BAC*pks* (Fig. 4C). The comet tail moment increased with the number of infecting DH10B BAC*pks* bacteria (Fig. 4D), indicating increased amounts of DSBs.

E. coli strains harboring a genomic island, widely distributed in both pathogenic and com-

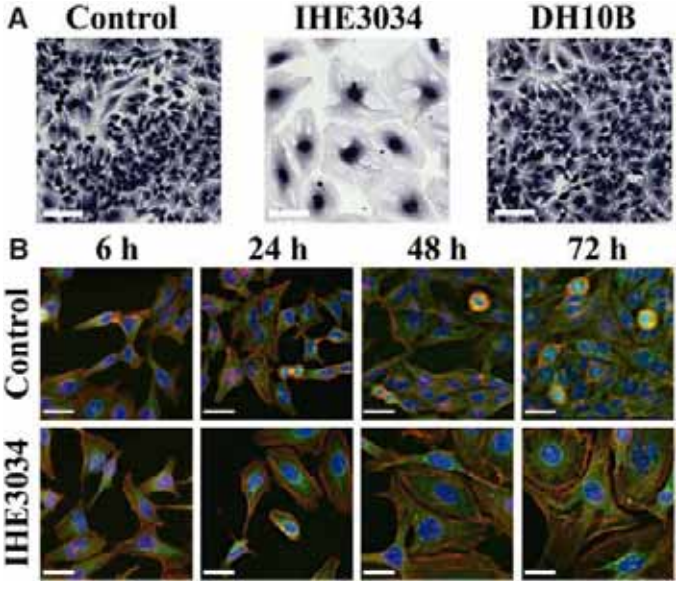

Fig. 1. Morphologic changes were induced upon transient infection of epithelial cells with *E. coli*. (**A**) Live pathogenic *E. coli* strain IHE3034 or laboratory strain DH10B was added directly onto HeLa cells, cocultivated for 4 hours, then washed. The cells were incubated for 72 hours with gentamicin before staining with Giemsa. Scale bars, 100 μm. (**B**) Cell nuclei (blue), F-actin (red), and α-tubulin (green) demonstration 6 to 72 hours after transient infection with IHE3034 (bottom) or without IHE3034 (top). Scale bars, 40 μm.

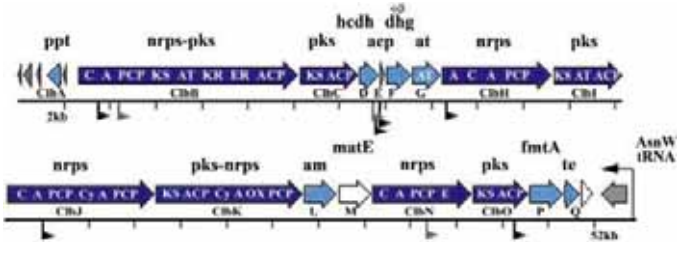

Fig. 2. Schematic map of the 54-kb *pks* island. Localization of transposon insertions in strains IHE3034 and SP15 resulting in loss of the cyopathic effect are indicated by black and gray flags. Open reading frames (ORFs) whose gene products are involved in peptide-polyketide synthesis and cytopathic effect are indicated in different shades of blue (NRPS and PKS, dark blue; others, light blue). ORFs not strictly required for the cytopathic effect are shown in white. Transposase and integrase ORFs are shown in gray. ORF designations are given below the ORF symbols. Clb, colibactin. The predicted functions are shown above the ORF; ppt, phosphopantetheinyl transferase; nrps, nonribosomal peptide synthetase; pks, polyketide synthase; hcdh, hydroxyl acyl coA dehydrogenase; acp, acyl carrier protein; dhg, αβ dehydrogenase; at, acyl-transferase; am, amidase; te, thioesterase. The predicted domain organization of NRPS and PKS is indicated: A, adenylation; ACP/PCP, phosphopantetheine/acyl carrier; AT, acyltransferase; C, condensation; Cy, cyclization; ER, enoyl reductase; KR, ketoacyl reductase; KS, ketoacyl synthase; OX, oxidation.

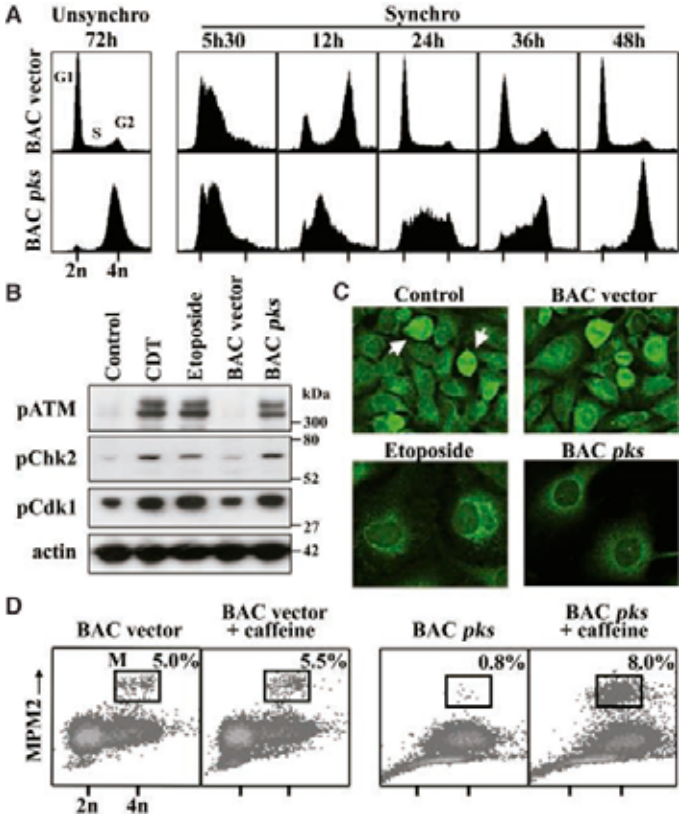

Fig. 3. Transient infection with *E. coli* harboring the *pks* island induced cell cycle arrest and activation of the G_2 checkpoint. (**A**) HeLa cells were synchronized in G_1/S (Synchro) or left unsynchronized (Unsynchro), then infected 4 hours with DH10B harboring the BAC*pks* or the vector alone. Cell cycle progression was monitored by flow cytometry at given times after infection. (**B**) G_1/S-synchronized HeLa cells were infected as before and the activation of the DNA damage pathway was examined 48 hours after infection by Western blotting, with the use of antibodies that recognize the phosphorylated (p) forms of target proteins. As controls, cells were treated with etoposide and purified Cytolethal Distending Toxin (CDT), both known to activate the DNA damage cascade response. kDa, kilodaltons. (**C**) Cells were infected as in (B) and intracellular localization of Cdc25C was observed by confocal microscopy. Note Cdc25C cytoplasmic sequestration in giant cells, whereas in controls Cdc25C was found in nuclei of dividing cells (arrows). (**D**) G_1/S-synchronized HeLa cells were infected, incubated for 42 hours, and further treated with or without caffeine for 6 hours. Cell cycle distribution was analyzed by bivariate flow cytometry for DNA content and mitotic phosphoproteins (MPM-2) to discriminate mitotic cells from G_2 cells in the 4n population. Percentages of mitotic cells are shown.

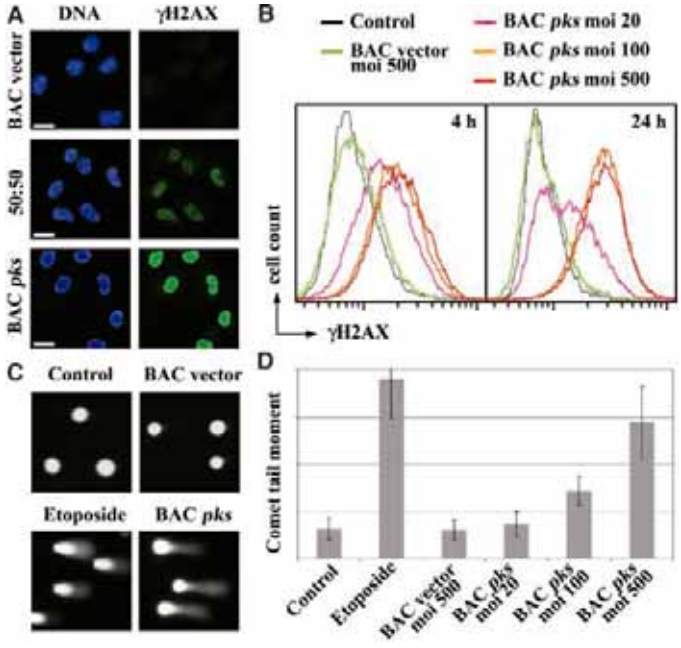

Fig. 4. Exposure to *E. coli* harboring the *pks* island induced host DNA DSBs. (**A**) HeLa cells were infected with DH10B harboring the BAC*pks* or empty vector [multiplicity of infection (moi) = 100] or with a 50:50 mix of each strain and examined 4 hours later for DNA (blue) and for phosphorylated H2AX (γH2AX) (green). Scale bars, 20 μm. (**B**) HeLa cells were infected 4 hours with given moi, then 4 to 24 hours later, γH2AX was quantified by flow cytometry. (**C**) HeLa cells were infected or treated with etoposide, then embedded in agarose, lysed, and subjected to an electric field in neutral condition that allowed migration of broken DNA out of nuclei (comet assay). DNA was stained and examined by fluorescence microscopy. (**D**) Cells were infected as in (B), the comet assay was performed, and the mean comet tail moment (product of tail length and fraction of DNA in the tail) was measured. The error bars represent the standard error of the mean.

mensal isolates, induce DSBs upon transient contact with epithelial cells (fig. S7). This genomic island is present in Nissle 1917, a commensal strain of *E. coli* that is an excellent colonizer in mice and humans and has been widely used as a probiotic treatment for intestinal disorders, such as ulcerative colitis and Crohn's disease (*22*–*24*). Slowing the renewal of the intestinal epithelium by blocking the cell cycle could be a bacterial strategy to prolong colonization of the intestinal epithelium, which in turn should have an impact on pathogenicity and commensalism (*12*). The amount of the genotoxin produced by different strains together with the location and duration of the contact with the target host cells may be critical to whether commensalism or pathogenicity is promoted. Because DNA DSBs can give rise to genomic instability (*25*), the occurrence of bacteria with the *pks* island may also constitute a predisposing factor for the development of intestinal cancer (*26*). Friend or foes, synthesis of a bioactive polyketide-peptide in *E. coli*, the workhorse organism for genetic engineering, should facilitate progress in engineering hybrid peptide-polyketide biosynthetic pathways for making natural products such as anticancer agents, antibiotics, and immunosuppressants (*27*, *28*).

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Tequila, a Neurotrypsin Ortholog, Regulates Long-Term Memory Formation in *Drosophila*

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Mutations in the human neurotrypsin gene are associated with autosomal recessive mental retardation. To further understand the pathophysiological consequences of the lack of this serine protease, we studied Tequila (Teq), the *Drosophila* neurotrypsin ortholog, using associative memory as a behavioral readout. We found that *teq* inactivation resulted in a long-term memory (LTM)–specific defect. After LTM conditioning of wild-type flies, *teq* expression transiently increased in the mushroom bodies. Moreover, specific inhibition of *teq* expression in adult mushroom bodies resulted in a reversible LTM defect. Hence, the Teq pathway is essential for information processing in *Drosophila*.

Mental retardation (MR) is the most common handicap in children and young adults, affecting 1 to 3% of the population. The causes of MR are diverse, and genetic and metabolic diseases account for about one-third of cases. Understanding the mechanisms of MR has long been hampered by both the complexity and the heterogeneity of these conditions. This is particularly true for nonsyndromic MRs (i.e., MR with apparently normal brain development and no other clinical features). A mutation in the human neurotrypsin gene (*PRSS12*) has been reported in nonsyndromic MR (*1*). Neurotrypsin is a multidomain neuronal trypsin-like serine protease predominantly expressed in the developing and adult nervous system (*2*). Neurotrypsin might be involved in synaptic development (*2*). However, its exact function remains elusive.

Progress in *Drosophila melanogaster* genetics and similarities between human and fly genomes have made comparative approaches feasible (*3*). MR-associated molecules are remarkably well conserved across the two species; 87% of the genes involved in MR have a fly ortholog. Moreover, in 76% of the cases, the extent and type of amino acid sequence similarities suggest similar functions (*3*). Thus, neurotrypsin and the only *Drosophila* ortholog, Teq, show a high degree of amino acid conservation, particularly in the region of the functional domains (fig. S1).

Whether the cognitive disorders caused by neurotrypsin mutations are due to improper brain maturation or to a primary plasticity defect during information processing is uncertain. To address this issue, we studied the involvement of *teq* in long-term memory (LTM). We used classical conditioning of an odor-avoidance response. In this paradigm, the flies are exposed

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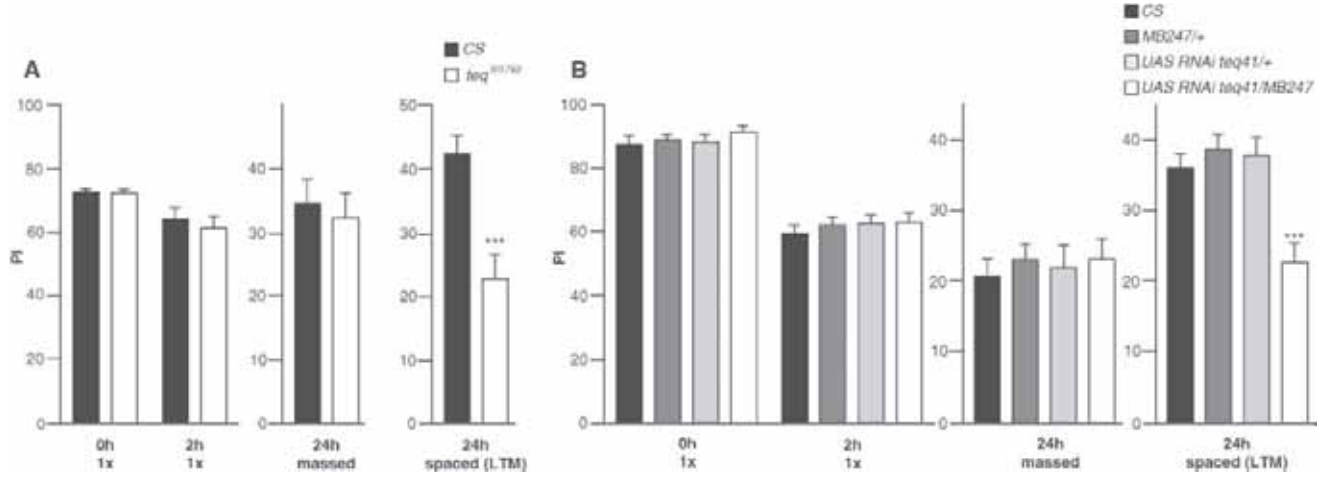

Fig. 1. *teq*^{f01792} mutants show an LTM-specific defect. (**A**) *teq*^{f01792} is an LTM-specific mutant. Performance indices (PIs) were measured at 0 hours (n = 10 groups) and 2 hours (n = 18 or 19 groups) after a single conditioning cycle, 24 hours after massed (n = 13 or 14 groups) or spaced training (n = 14 to 16 groups). Results are means ± SEM; ***$P < 0.001$ (t test). (**B**) Inhibition of *teq* expression in the MBs leads to a LTM-specific defect. The *MB247* Gal4 line shows expression in neurons that project to all MB lobes. PIs were measured at 0 hours (n = 10 groups), 2 hours (n = 35 groups) after a single conditioning cycle, and 24 hours after massed (n = 20 groups) or spaced training (n = 29 to 33 groups). Results are means ± SEM; ***$P < 0.001$ (t test).

to two distinct odors, one of which is accompanied by an electric shock (*4*). With repeated and spaced training bouts, LTM is formed that is dependent on new protein synthesis (*5*). In the absence of rest intervals between training sessions ("massed training"), a distinct form of memory is produced that does not require protein synthesis (*5*). A *piggyBac* insertion in the *teq* gene (referred to as teq^{f01792}) (*6*) was shown to decrease Teq expression (fig. S2). The mutation was first outcrossed over 10 generations to shift its genetic background to that of the reference strain *Canton-Special* (*CS*). Interestingly, teq^{f01792} displayed a decrease in 24-hour LTM after spaced training, whereas a normal 24-hour memory capacity was observed after massed training (Fig. 1A). After a single conditioning, teq^{f01792} learning and 2-hour memory were also normal, showing that teq^{f01792} is a LTM-specific mutant.

The mushroom bodies (MBs) are bilateral symmetrical structures of the *Drosophila* brain (*7*) (Fig. 2B) essential for olfactory learning and memory. MBs play a key role in LTM (*8*, *9*). We therefore examined the integrity of these structures in the *teq* mutant by immunohistochemistry using antibodies to fasciclin II (*10*) or to protein kinase A catalytic subunit (*11*) and paraffin sections (figs. S3 and S4). No structural defect was detected in MBs, which suggested that the teq^{f01792} mutation does not impair LTM via an abnormal development of MBs. However, more subtle developmental defects may not have been detected at this level of resolution.

To specifically silence the *teq* gene in the MBs, we took advantage of the GAL4-UAS system combined with RNA interference (RNAi) (*12*). We generated transgenic flies expressing a *teq* RNAi construct under the control of

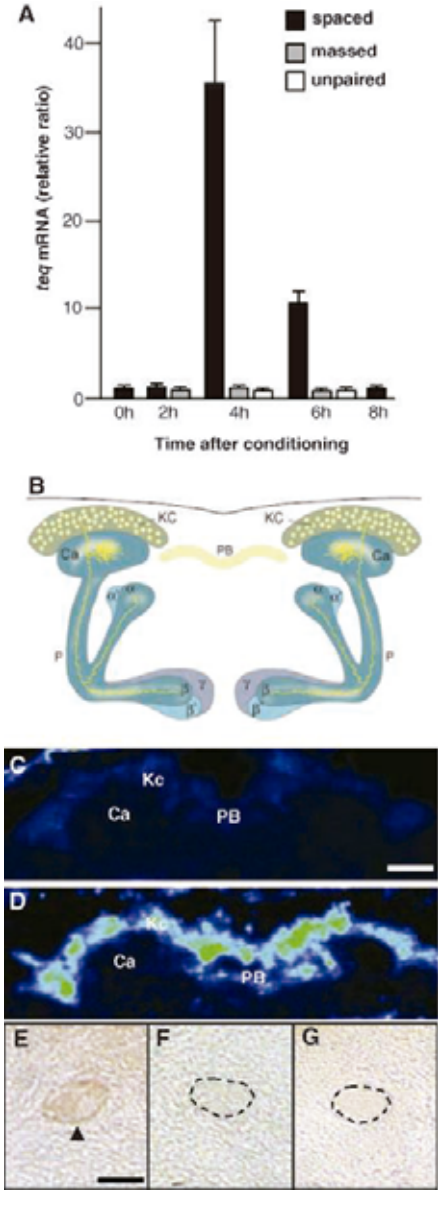

Fig. 2. *teq* mRNA expression is up-regulated after LTM conditioning. (**A**) The level of head *teq* mRNA is up-regulated after LTM conditioning. Heads of *CS* flies were collected at different times after spaced training, total RNA was extracted, and quantitative RT-PCR was performed. Quantitative RT-PCR experiments indicate that the level of *teq* RNA is up-regulated from 4 to 6 hours after spaced training (n = 4 to 9 groups). No significant changes were observed after massed (n = 5 to 7 groups) or unpaired training (n = 4 to 6 groups). The ratio represents [*teq* mRNA (trained)/*tub* mRNA (trained)]/[*teq* mRNA (naïve)/*tub* mRNA (naïve)]. (**B**) Schematic representation of the adult *Drosophila* MBs. Each of the MBs comprises about 2500 parallel-packed neurons that are organized into distinct computational networks. The MB cell bodies (Kenyon cells, KC) are located at the dorsal cortex, extending their dendrites into the calyx (Ca), which receives olfactory information from the antennal lobes. More distally, MB axons project to the anterior portion of the brain via a dense structure known as the peduncle (P), where they give rise to five major lobes (α, α′, β, β′, and γ) (*6*). PB, protocerebral bridge. (**C** and **D**) FISH of *CS* brain sections at the protocerebral bridge level. Each probed slide carried a mixture of brains from naïve (C) and trained flies (D) to ensure identical treatment. *teq* mRNA is expressed in Kenyon cells 4 hours after conditioning. Scale bar, 50 μm. (**E** to **G**) Teq immunostaining on brain sections at the peduncle level. Five hours after the end of the training, Teq is detected in the MB peduncle (arrowhead) of the conditioned flies (E), whereas no staining is detected in conditioned teq^{f01792} mutant (F) or naïve *CS* flies (G). Dotted lines outline the peduncle limits. Scale bar, 20 μm.

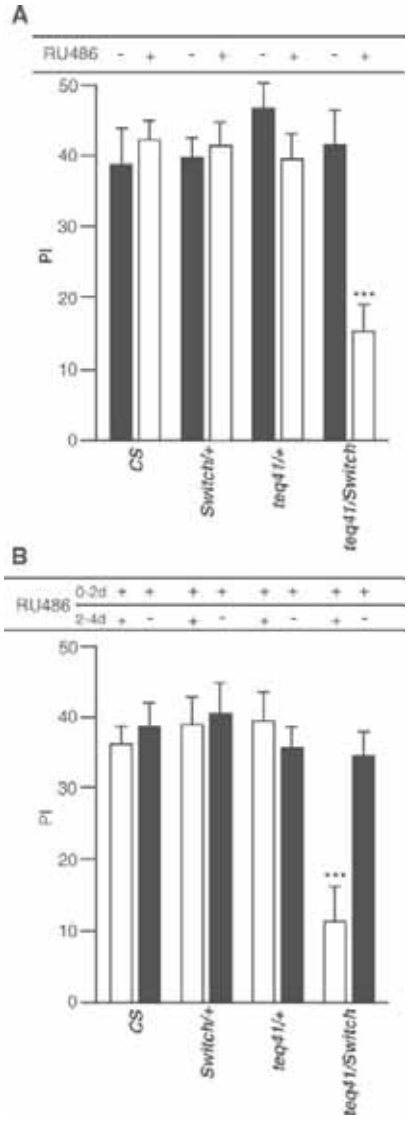

Fig. 3. *teq* is acutely required for LTM formation. (**A**) Expression of *teq* RNAi in adult MBs leads to an LTM defect. PIs were measured 24 hours after spaced training (n = 11 to 14 groups). Flies were fed during 2 days with food supplemented with 200 μM RU486. There was no effect of RU486 administration on LTM of control flies. Results are means ± SEM. ***$P < 0.001$ (t test) with the appropriate genetic control: *UAS RNAi teq 41/MB247-Switch* (+RU486) versus *UAS RNAi teq 41/MB247-Switch* (–RU486). (**B**) LTM impairment in conditional *teq* mutant is reversible. Flies were fed during 2 days with food supplemented with 200 μM RU486 to induce *teq* RNAi expression. After this period, flies were transferred onto regular food or food with RU486. Flies recovered a normal LTM capacity after 2 days without RU486, whereas continuous expression of the *teq* RNAi led to the typical LTM defect. PIs were measured 24 hours after a spaced training (n = 15 to 18 groups). Results are means ± SEM. ***$P < 0.001$ (t test) with the appropriate genetic control: *UAS-RNAi teq 41/MB247-Switch* (–RU486) versus *UAS-RNAi teq 41/MB247-Switch* (+RU486).

MB247, a specific GAL4 driver of larval and adult MBs (*4*, *13*). RNAi-mediated *teq* knockdown induced a decrease in 24-hour LTM, whereas 24-hour memory after massed training was unaffected, as were learning and 2-hour memory after single conditioning (Fig. 1B). This effect was found to be independent of the insertion site of the RNAi construct (fig. S5A). Thus, the inactivation of *teq* in the MBs resulted in a LTM-specific defect similar to that induced by the *piggyBac* constitutive mutation. Similar LTM defects were observed with two additional MB GAL4 drivers, Gal5122 (*14*) and 238Y (*15*) (fig. S5B). As expected, no alteration of MB morphology was observed in *Gal4/UAS-RNAi teq* mutants (figs. S3 and S4).

Because LTM formation requires de novo protein synthesis that depends partly on transcriptional regulation (*5*, *14*, *16*), we investigated whether *teq* expression was regulated after LTM conditioning in the wild-type fly. Head RNAs were extracted at various times after spaced training, and levels of *teq* mRNA were assayed by quantitative reverse transcription polymerase chain reaction (RT-PCR) (*4*). The *teq* mRNA level was increased 4 to 6 hours after the end of LTM training (Fig. 2A), whereas no change was observed 2 or 8 hours after training. No variation in *teq* expression was observed in flies subjected to massed training, nor in pseudo-conditioned flies that received the odor and electric shock stimuli in a temporally dissociated manner, a protocol that does not induce learning (*5*) (Fig. 2A).

To identify brain structures in which the *teq* gene was overexpressed, we performed fluorescence in situ hybridization (FISH) on brain sections after a spaced training (*4*). *teq* mRNA was strongly expressed in MB cells 4 hours after LTM training, whereas it was weakly expressed in naïve flies (Fig. 2, C and D). The time course of the gene expression observed in situ correlated with that observed after quantitative RT-PCR. In a second step, polyclonal antibodies were raised against Teq protein. Five hours after LTM training, the Teq protein was detected by antibodies to Teq in the MB peduncles (*4*) (Fig. 2E). No noticeable staining was found in the peduncle of naïve wild-type flies or in trained teq^{f01792} flies (Fig. 2, F and G). A signal was observed in the cortical region of the MB in naïve and trained CS flies (fig. S6). It is noteworthy that Teq expression was observed neither in the dendritic region of the MBs nor in MB lobes in naïve or trained CS flies (fig. S6).

The observation that *teq* expression is up-regulated in MBs after LTM conditioning strongly suggests that Teq is physiologically involved in brain plasticity. To further support this hypothesis, we used the inducible Gene-Switch system (*17*). In this system, the DNA binding domain of the GAL4 protein is fused to the progesterone receptor to generate the RU486-inducible chimeric activator. In the absence of RU486, the Gene-Switch is in the "off" state. In the presence of hormone, the chimeric protein undergoes a conformational change and it can bind to a UAS sequence and activate transcription of the RNAi construct. Specific expression of *teq* RNAi in adult MBs led to a strong LTM deficit (Fig. 3A and fig. S5C) with no obvious structural anomalies in the MBs (fig. S3).

To further study the dynamics of *teq* involvement in this process, we addressed the question of whether expressing Teq in a previously Teq-defective fly restored normal LTM capacity. Hence, *teq* RNAi was first induced for 2 days in adult MBs. *MB247-Switch/UAS-RNAi* flies were then transferred to food without RU486 to restore MB *teq* mRNA expression. These flies regained a normal 24-hour LTM capacity (Fig. 3B), thus demonstrating that the lack of Teq has reversible consequences for *Drosophila* brain function.

Several studies have emphasized a role of serine proteases in the nervous system (*18*, *19*). During neural development, serine proteases contribute to cell migration, axon outgrowth, and synapse elimination (*20*). In adult life, they play a role in neuropeptide processing, regulation of neuronal survival, and structural plasticity associated with learning and memory processes. Mentally retarded children with neurotrypsin mutations have normal milestones of psychomotor development over the first 18 months and become retarded starting at 2 years of age (*1*), suggesting a specific role of neurotrypsin in postnatal cognition processes (*20*). It is therefore important to determine whether their cognitive disorder is due to improper brain maturation at the ultrastructural level or to a physiological dysfunction. The results obtained with *Drosophila teq* support the view that the Teq pathway (and by analogy the neurotrypsin pathway in humans) is essential for information processing and functional plasticity because (i) *teq* mutants have a LTM-specific defect, (ii) *teq* mRNA is up-regulated during a short time window after spaced training, (iii) *teq* is specifically required in adult MBs, and (iv) impairment of LTM capacity after transient *teq* silencing is reversible. Further experiments will be required to determine the function of Teq within the adult MBs, in particular at the level of the peduncle.

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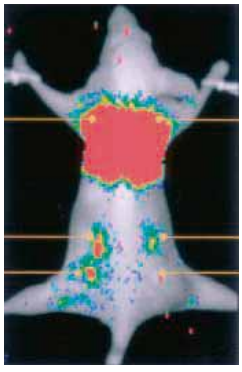

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Biomedical Communications

This senior level position at the Johns Hopkins Medical Institutions requires leadership skills and experience commensurate with direction of an office providing professional and career skills to medical and graduate students, postdoctoral fellows, and junior faculty at the Johns Hopkins Schools of Medicine, Public Health, and Nursing. The successful candidate must take responsibility for design, development, and teaching of classes and workshops in biomedical communications, emphasizing the preparation of successful grant submissions, the writing of research articles, and presentation of research seminars and educational lectures. The position also requires oversight of three staff members.

It is anticipated that the successful candidate will have an advanced degree (M.A. or Ph.D. or equivalent) and several years of experience teaching large classes and facilitating small group discussions at the graduate and postgraduate levels. An understanding of career and professional issues relevant to graduate students and postdoctoral fellows in the biomedical sciences is highly desired. Outstanding interpersonal skills are required, including the ability to relate to those from diverse cultures and to integrate with other institutional offices concerned with professional development. Johns Hopkins Medical School has a competitive salary program with outstanding benefits.

Please submit resumes/curriculum vitae to: **Dr. Peter Maloney, Associate Dean for Graduate Student Affairs, Johns Hopkins Medical School, 1830 Building, Room 2-108, 1830 East Madison Street, Baltimore, MD 21205.**

Johns Hopkins University is committed to policies of Equal Opportunity and Affirmative Action, which are essential to its mission of promoting research, service and academic excellence. The successful candidate for this position will be subject to a pre-employment background check.

FACULTY POSITION IN ORGANIC CHEMISTRY
University of California, Davis

The Chemistry Department **(website: http://www.chem.ucdavis.edu/)** at the University of California, Davis, invites applications for one faculty position at the **ASSISTANT PROFESSOR** level in organic chemistry. The preferred candidate will develop a strong research program in organic chemistry at the biological interface. A Ph.D. or equivalent in chemistry, medicinal chemistry, or pharmaceutical sciences is required. The candidate must also demonstrate a strong commitment to undergraduate and graduate teaching. This position is open until filled; but to assure full consideration, applications should be received no later than September 22, 2006. The targeted start date is July 1, 2007. Interested candidates should arrange for three letters of recommendation and both paper and electronic (PDF format) copies of curriculum vitae, publication list, teaching statement, and research plans to be sent to:

Organic Search Committee
Department of Chemistry
University of California at Davis
One Shields Avenue
Davis, CA 95616

The University of California is an Affirmative Action/Equal Opportunity Employer.

NIH-funded **RESEARCH ASSISTANT POSITION.** The project combines bioinformatic analysis with a forward genetic screen of Drosophila mutant fly lines to identify novel genes regulating feeding and fat deposition. Follow-up of corresponding mammalian homologs as candidate modulators of metabolic function will include biochemical and molecular pharmacological approaches in vitro and in vivo. Experience with molecular biology required, some knowledge of Drosophila is desirable. Contact: **Alan S. Kopin, Director, Molecular Pharmacology Research Center; e-mail: akopin@tufts-nemc.org.** *Tufts New England Medical Center is an Equal Opportunity Employer.*

POSITIONS OPEN

SYNAPTIC PLASTICITY/GLIAL-NEURONAL INTERACTIONS (ASSISTANT PROFESSOR). The Department of Cell Biology and Neuroscience **(website: http://cbns.ucr.edu/)** at the University of California (UC), Riverside, is strengthening its faculty in the area of synaptic plasticity/glial-neuronal interactions. Construction of a new laboratory building, the Biological Sciences Building, is now complete (as of June 2006) and will house the new faculty as well as most of those in the current Department. This search seeks applicants at the Assistant Professor level whose primary research interests are directed towards investigating signaling underlying synapse formation and plasticity. Applicants must hold a Ph.D., and postdoctoral experience is essential for candidates at the Assistant level. It is expected that this hire would interact broadly with faculty in our Neuroscience Graduate Program as well as Department faculty with interests in other areas of cell biology, and with members of the Center for Glial-Neuronal Interactions at UC Riverside. Additionally, this person could further strengthen our links with the Center for Nanoscale Science and Engineering and the stem cell focus. Opportunities for graduate student training are available through participation in several interdepartmental graduate programs, including the Neuroscience Program **(website: http://neuro.ucr.edu/)** and the Cellular, Molecular and Developmental Biology Program **(website: http://www.cell.ucr.edu/).** Other teaching responsibilities would be at the undergraduate level. Send applications, including curriculum vitae, personal research statement, and at least three letters of reference to: **Chair, Plasticity Search Committee, Department of Cell Biology and Neuroscience at the University of California, Riverside, CA 92521 U.S.A.** Review of applications will begin October 2, 2006, and will continue until the position is filled. *The University of California is an Equal Opportunity/Affirmative Action Employer.*

Biomanufacutirng Research Institute and Technology Enterprise (BRITE) Center for Excellence, a newly established institute, will provide training and research for B.S., M.S., and Ph.D. in pharmaceutical science. BRITE, located at North Carolina Central University (NCCU), is seeking applicants for **TENURE-TRACK POSITIONS** in microbial and protein group and bioanalytical chemistry group. Research is desirable in the optimization of the scale-up process using recombinant microorganisms and the downstream process of improvement of purification, covalent modifications and folding of active macromolecule. Methods development in time-of-flight mass spectrometry, high pressure liquid chromatography, and capillary electrophoresis is highly desirable.

A Ph.D. in biochemistry or a related discipline; a record of publication, grantsmanship, and independence are required. Industrial experience in process development is a plus. Candidates will be expected to develop or maintain a strong externally funded research program and contribute to enthusiastic teaching.

Applicants should submit curriculum vitae with publications, research interests, teaching experience, and three references. Review of applicants will begin immediately and continue until the position is filled. Contact information: **c/o Ms. Sharon Jordan** at **e-mail: sjordan@nccu.edu.** For more information about the BRITE/NCCU, please visit **website: http:// www.nccu.edu\BRITE.**

NCCU is an Affirmative Action/Equal Opportunity Employer.

CAREER OPPORTUNITY

This unique program offers the candidate with an earned doctorate in the life sciences the opportunity to obtain the Doctor of Optometry (O.D.) degree in 27 months (beginning in March of each year). Employment opportunities exist in research, education, industry, and private practice. Contact the **Admissions Office, telephone: 800-824-5526** at **The New England College of Optometry, 424 Beacon Street, Boston, MA 02115.** Additional information at **website: http://www.neco.edu, e-mail: admissions@neco.edu.**

Chief, Laboratory of Bacterial Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR) is seeking an outstanding individual to head the newly established Laboratory of Bacterial Diseases (LBD) in Bethesda, Maryland. The laboratory is to be located in the new C.W. Bill Young Center for Biodefense and Emerging Pathogens located on the NIH campus in Bethesda, Maryland.

The mission of the LBD will be to study basic and applied aspects of bacterial diseases related to biodefense or emerging and re-emerging pathogens, focusing on pathogenic bacteria. Exceptional scientists with research interests in basic, translational or clinical aspects of bacterial pathogenesis are encouraged to apply. The long-term goals of the Institute include supporting research that enables the development of new diagnostics, vaccines, and therapeutics.

This position requires an M.D., Ph.D. or equivalent with proven leadership abilities and a strong independent research program. Preference will be given to candidates with a documented record of accomplishment in bacterial disease research, and those whose program(s) are consistent with the mission of the NIAID.

The Laboratory Chief will have independent resources to conduct basic and clinical research and will supervise other Principal Investigators with independent research programs. The successful candidate is expected to lead a strong research program in laboratory and/or clinical research. Committed resources include space, support personnel and an allocated annual budget to cover service, supplies, animals and related resources and salaries. A Laboratory Chief in the DIR is equivalent to a Department Chair in a University or Medical School. Applicants must be U.S. citizens or permanent residents and be eligible for the appropriate security clearance under the CDC Select Agent Program. Salary will be commensurate with experience and qualifications.

Interested candidates may contact **Dr. Karyl Barron, Deputy Director, DIR, NIAID at 301/402-2208 or email (kbarron@niaid.nih.gov)** for additional information about the position and/or infectious diseases research at the NIH.

To apply for the position, candidates must submit curriculum vitae, bibliography, a detailed statement of research interests, and reprints of up to three selected publications, preferably via Email to: Lynn Novelli at novelly@niaid.nih.gov. In addition, the names of three potential references must be sent to **Dr. Steven M. Holland, Chair, NIAID Search Committee, c/o Ms. Lynn Novelli, DIR Committee Manager, 10 Center Drive, MSC 1356, Building 10, Room 4A26, Bethesda, Maryland 20892-1356.** Completed applications **MUST** be received by **Monday, September 25, 2006**. Please refer to **AD#004** on all correspondence. Further information on this position and guidance on submitting your application is available on our website at: **http://healthresearch.niaid.nih.gov**

ANNOUNCEMENT OF A NIH ROADMAP RESEARCH FUNDING OPPORTUNITY

Assay Development for High Throughput Screening

***Request for applications* RM-07-001**

This RFA is one component of the NIH Roadmap Molecular Libraries and Imaging Initiative **(http://nihroadmap.nih.gov/molecularlibraries/)**. Its goal is to stimulate pharmacological probe design by funding development of scientifically novel and technologically robust assays that can be miniaturized, automated, and used to interrogate small molecule libraries. Investigators are asked to state a biological question that can be addressed through the use of a pharmacological small molecule probe, to further identify the requisite features that should be encompassed in its design, and develop a screening plan of assays that can be used to identify small molecules with essential probe attributes.

Assay development proposals should aim to develop assay protocols for novel molecular targets and phenotypes and transition them to a *high throughput screening* format. Investigators should additionally define and characterize a screening project plan to include secondary and counter-screening assays. Emphasis will be placed on screening targets for which an inadequate array of selective and potent small molecule modulators are available to the public. Support will be provided via a 1-year R21 ($125,000 available in direct costs).

The RFA is intended to promote the development of automated screening projects that are eligible to enter the Roadmap Molecular Libraries Screening Center Network. Funded applications will be able to directly request screening by the MLSCN following project completion. The overall goal of the Molecular Libraries and Imaging Initiative is to create a public database of biological information about small molecule chemical structures **(see PubChem; http://pubchem.ncbi.nlm.nih.gov)**, which will further seed the development of small molecule pharmacological tools for biological research.

It is anticipated that 40-50 projects will be funded (for $8 million) in response to two announcement dates, with the first occurring this Fall. Further announcements are planned in succeeding years. **Investigators should submit a letter of intent by September 8 for the next submission date of September 22, 2006**. Additional information about the announcement can be obtained at the following website: **http://grants.nih.gov/grants/guide/rfa-files/RFA-RM-07-001.html, or, by contacting Program Director Mark Scheideler, Ph.D., by email at: scheidelerm@ninds.nih.gov**.

WWW.NIH.GOV

Chief, Laboratory of Virology
National Institute of Allergy and Infectious Diseases
National Institutes of Health

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR) is seeking an outstanding individual to head the newly established Laboratory of Virology (LV) located at the Rocky Mountain Laboratories in Hamilton, Montana. LV will interact with four other Intramural Research Laboratories at this location presently studying infectious diseases involving viruses, bacteria, rickettsia, chlamydia and prions.

The mission of the LV is to study high containment BSL-3 and BSL-4 viral pathogens with the goal of developing diagnostics, vaccines, and therapeutics. The research to be conducted in the LV is to include studies of vector/reservoir transmission, pathogenesis, pathophysiology and host immune response of high containment viral pathogens. In addition, the LV must maintain a flexible infrastructure to permit rapid analysis of newly emerging high containment viral pathogens of special interest.

The selected candidate will supervise research in a newly constructed Integrated Research Facility which houses three BSL-4 lab suites, three BSL-3 lab suites and multiple BSL-2 lab suites, as well as extensive associated BSL-2, 3, and 4 animal facilities.

This position requires a Ph.D., M.D., D.V.M. or equivalent with proven ability to carry out a strong independent research program. Preference will be given to candidates with a record of leadership and accomplishment in BSL-4 or Select Agent BSL-3 viral pathogen research, with program(s) consistent with the mission of the NIAID. The selected person will also be expected to recruit and supervise other Principal Investigators with independent research programs.

The Laboratory Chief will have independent resources to conduct laboratory research and translational/clinical research, as appropriate. Committed resources include space, support personnel and an allocated annual budget to cover service, supplies and salaries. A Laboratory Chief in the DIR is equivalent to a Department Chair in a University or Medical School. Applicants must be eligible for the appropriate security clearance under the CDC Select Agent Program. Salary is dependent on experience and qualifications. Interested candidates may contact **Dr. Karyl Barron, Deputy Director, DIR, NIAID at 301/402-2208 or email (kbarron@niaid.nih.gov)** for additional information about the position.

To apply for the position, candidates must submit a curriculum vitae, bibliography, a detailed statement of research interests, the names of three references, and reprints of three selected publications, preferably via email to: **Felicia Braunstein at braunsteinf@niaid.nih.gov or by US Mail to: Ms. Felicia Braunstein, DIR Committee Manager, 10 Center Drive MSC 1349, Building 10, Rm. 4A-30, Bethesda, Maryland 20892-1349**. Please note search **#006** when sending materials. Completed applications **MUST** be received by **Friday, November 3, 2006**. Further information on working at NIAID is available on our website at: **http://healthresearch.niaid.nih.gov**

Chief, Laboratory of Human Bacterial Pathogenesis
National Institute of Allergy and Infectious Diseases
National Institutes of Health

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR) is seeking an outstanding individual to head the Laboratory of Human Bacterial Pathogenesis (LHBP) in Hamilton, Montana.

The mission of the LHBP is to study human bacterial diseases related to emerging and re-emerging pathogens. The research to be conducted in the LHBP is to include; 1) the molecular basis of host-pathogen interactions, 2) the genetic basis of bacterial virulence and pathogenesis, 3) the use of animal modeling to define host defense mechanisms and biology and immunology of host-pathogen interactions, and 4) development of novel and improved intervention strategies to control bacterial infectious diseases. The ultimate goal is to develop diagnostics, vaccines, and therapeutics for emerging and re-emerging infectious diseases.

This position requires a Ph.D. and/or M.D. or equivalent with proven leadership abilities and a strong independent research program. Preference will be given to candidates with a documented record of accomplishment in bacterial disease research, and especially to those whose program(s) are consistent with the mission of the NIAID to study emerging and re-emerging bacterial pathogens.

The Laboratory Chief will have independent resources to lead and conduct laboratory research and translational/clinical research, as appropriate. Mechanisms are available to conduct clinical studies at the Bethesda campus and/or to obtain clinical samples through contract mechanisms at non-NIH institutions. The individual will supervise other Principal Investigators with independent research programs investigating the pathogenicity of Staphylococcus and Streptococcus species. Committed resources include space, support personnel, animal resources and an allocated annual budget to cover service, supplies and salaries. A Laboratory Chief in the DIR is equivalent to a Department Chair in a University or Medical School. Salary is dependent on experience and qualifications.

Interested candidates may contact **Dr. Karyl Barron, Deputy Director, DIR, NIAID at (301) 402-2208 or email (kbarron@niaid.nih.gov)** for additional information about the position. To apply for the position, candidates must submit a curriculum vitae, bibliography, a detailed statement of research interests, and reprints of up to three selected publications preferably via email to: **Felicia Braunstein at braunsteinf@niaid.nih.gov or by US Mail to: Ms. Felicia Braunstein, DIR Committee Manager, 10 Center Drive MSC 1349, Building 10, Rm. 4A-30, Bethesda, Maryland 20892-1349**. In addition, the names of three referees must be sent to **Dr. Tom Schwan, Chairperson, NIAID Search Committee, c/o Ms. Felicia Braunstein, DIR Committee Manager, 10 Center Drive MSC 1349, Building 10, Rm. 4A-30, Bethesda, Maryland 20892-1349**. Please note search **#005** when sending materials. Completed applications **MUST** be received by **October 6, 2006**. Further guidance on submitting your application is available on our website at: **http://healthresearch.niaid.nih.gov**.

Director, Division of Cardiovascular Diseases
National Heart, Lung, and Blood Institute

The National Heart Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) seeks a dynamic physician-scientist to provide strategic leadership for its newly organized Division of Cardiovascular Diseases (DCVD). The Director will assume responsibility for creating and nurturing internationally-renowned programs which will participate actively in international research in cardiovascular diseases across the spectrum of basic science and clinical research including translational research and the conduct of a wide variety of clinical trials. The Director will recruit scientists and scientific administrators, develop and nurture a strong workforce, and build depth in disease-specific branches. Key challenges include establishment of priorities, integration of basic and clinical science, building teams, and interaction with scientific colleagues in many settings. Functioning as a key member of the senior leadership team of the Institute, the incumbent will collaborate with closely aligned programs in the Institute. The DCVD Director will have a profound impact upon the national investment in research, and the quality of service to the international research community. The Director of DCVD will have the opportunity to advocate for areas of critical importance to the national and global populace, to establish and implement programs congruent with NHLBI's strategic plan, and to improve the health of the public. Applicants must possess an MD or equivalent degree as well as senior level research experience, interpersonal and communications expertise and ability. The successful candidate will be a respected, accomplished researcher with maturity, integrity and outstanding communication skills.

Application Process: Please submit your CV, bibliography, and two letters of recommendation to: **Joanna Fesler, Program Manager, STG International, Inc, 4900 Seminary Rd., Suite 1100, Alexandria, VA 22311**. For further information, please call **877-784-6452 or email jfesler@stginternational.com**. Your application package should be received by **October 15, 2006**. All information provided by candidates will remain strictly confidential and will not be released outside the NHLBI search process without a signed release from candidates.

Salary is commensurate with experience and a full package of Civil Service benefits is available including retirement, health and life insurance, leave and savings plan (401K equivalent).

The National Heart, Lung, and Blood Institute (NHLBI) provides leadership for a national program in diseases of the heart, blood vessels, lung, and blood; blood resources; and sleep disorders. With nationwide responsibility for improving the health and well-being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the NIH. The NIH encourages the application and nomination of qualified women, minorities and individuals with disabilities.

Director, Division of Prevention and Population Sciences
National Heart, Lung, and Blood Institute

The National Heart Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) seeks a dynamic scientist to provide strategic leadership for its newly reorganized Division of Prevention and Population Sciences (DPPS). The Director will assume primary responsibility for creating, nurturing and supporting internationally-renowned programs in population sciences and prevention in the areas of cardiovascular disease, and collaborating with closely aligned programs in the Division of Cardiovascular Diseases, Division of Lung Diseases, the Division of Blood Diseases and Resources, and the Division of Extramural Research Activities. The NHLBI also manages a strong program in biostatistics and resources for the conduct of clinical research. The NHLBI is engaged in a strategic planning process which will guide its scientific agenda for the next decade. The DPPS Director will advocate for areas of profound importance to the national and global populace, to establish and implement programs congruent with NHLBI's strategic plan and which will impact the health of the public. Functioning as a key member of the senior leadership team of the Institute, the incumbent will have a profound impact upon the national investment in research and the quality of service to the international research community. Applicants must possess an M.D., Ph.D. or equivalent degree as well as senior level research experience and ability to interact successfully with a broad range of individuals. The successful candidate will be a respected, accomplished scientist with maturity, integrity and outstanding communication skills.

Application Process: Please submit your CV, bibliography, and two letters of recommendation to: **Joanna Fesler, Program Manager, STG International, Inc, 4900 Seminary Rd., Suite 1100, Alexandria, VA 22311**. For further information, please call **877-784-6452 or email jfesler@stginternational.com**. Your application package should be received by **October 15, 2006**. All information provided by candidates will remain strictly confidential and will not be released outside the NHLBI search process without a signed release from candidates.

Salary is commensurate with experience and a full package of Civil Service benefits is available including retirement, health and life insurance, leave and savings plan (401K equivalent).

The National Heart, Lung, and Blood Institute (NHLBI) provides leadership for a national program in diseases of the heart, blood vessels, lung, and blood; blood resources; and sleep disorders. With nationwide responsibility for improving the health and well-being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the NIH. The NIH encourages the application and nomination of qualified women, minorities and individuals with disabilities.

Our work is someone's hope. Join us

in West Point, PA

NEUROSCIENCE
- Sr. Research Fellow
  Alzheimer's Disease
- Sr. Research Biologists
  Sleep and Pain
- Bio/Staff Bio/Research Biologists
  In vitro/in vivo Neurochemistry
  Sleep, Pain, Schizophrenia,
  Alzheimer's Disease, Stroke
- Bio/Staff Bio/Research Biologists
  In vivo - Alzheimer's Disease

VACCINES AND BIOLOGICS
- Sr. Research Biologist
  Molecular Virology
- Bio/Staff Bio/Research Biologists
  RNAi & Molecular Biology

CANCER RESEARCH
- Sr. Research Biologist
  RNAi

IMAGING
- Sr. Research Chemist
  Radiochemists
- Sr. Research Pharmacologists
- Bio/Staff Bio/Research Biologist
  - Pharmacologist
- Sr. Research Physicists
  MRI/MRS

STRUCTURAL BIOLOGY
- Sr. Research Chemist
- Bio/Staff Bio/Research Biologists

MOLECULAR ENDOCRINOLOGY
- Director
  Osteoporosis
- Sr. Research Biologist
  Sarcopenia

in Rahway, NJ

MEDICINAL CHEMISTRY
- Research Associate
  Synthetic Chemistry

TARGET VALIDATION
- Sr. Research Chemists

APOPTOSIS
- Sr. Research Scientists
- Research Scientists

IMMUNOLOGY
- Sr. Immunologists

METABOLIC DISORDERS
- Electrophysiologist

CARDIOVASCULAR BIOMARKERS
- Sr. Research Scientists
- Research Scientists

EXPERIMENTAL MEDICINE
- Assoc. Director, Clinical Research

CLINICAL PHARMACOLOGY
- Assoc. Director, Clinical Research

in Boston, MA

NEUROSCIENCE
- Sr. Director, Pharmacology
- Director, CNS Pharmacology
- Sr. Research Fellow
  Alzheimer's Disease
- Research Fellows
  Alzheimer's Disease
  CNS Pharmacology
- Sr. Research Biologists
  Alzheimer's Disease
  CNS Pharmacology
  Behavioral Pharmacology
- Research Associates
  Alzheimer's Disease
  CNS Pharmacology

MOLECULAR ONCOLOGY
- Research Biologists

RESEARCH OPERATIONS
- Research Animal Specialists

CANCER BIOLOGY AND THERAPEUTICS
- Research Associates

MEDICINAL CHEMISTRY
- Sr. Medicinal Chemist
- Sr. Computational Chemist
- Associate Scientists
  Medicinal Chemistry
  Drug Metabolism
  Synthetic Organic Chemistry

AUTOMATED LEAD OPTIMIZATION
- Lab Robotics/Automation Engineer
- Sr. Research Biochemist
  Focused Library Screening
- Sr. Research Biologist
  Physiochemical Screening

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University of Zurich

The Faculty of Science of the University of Zurich invites applications for the position of

Assistant Professor of Organic Chemistry

The ideal candidate will be someone at the start of his/her academic career, with an exceptional track record, and research interests in any area of organic chemistry, preferably in organic synthesis in its broadest sense. The successful person will be expected to establish an internationally recognized program of research, and participate in teaching organic chemistry within the Institute. The appointment will initially be non-tenured, with the possibility to obtain tenure after review.

The Institute of Organic Chemistry is part of the Chemistry Department at the University of Zurich. It is situated on the Irchel science campus, close to the center of Zurich where the University Hospital and medical institutes are located (see also http://www.oci.unizh.ch). Near by are the science Departments of the ETH Zurich. This concentration of science institutes provides a stimulating and attractive environment for interdisciplinary research.

The new Professor's appointment should commence by summer 2007, or as soon as possible thereafter. Applications received by September 15th will be guaranteed full consideration, but the committee is open to late applications of exceptional quality and will search until the position is filled. Applications, including a curriculum vitae, list of publications, outline of current and future research plans, and names of potential referees, should be sent to the Dean of the Science Faculty at the following address: Professor D. Wyler, Dekanat der Mathematisch-Naturwissenschaftlichen Fakultät, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. The CV and list of publications should also be submitted in a single PDF-file to jobsmnf@zuv.unizh.ch.

All enquiries should be directed to Prof. Jay Siegel, jss@oci.unizh.ch, Organic Chemistry Institute, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich. The University of Zurich is an equal opportunity employer. Applications from women are particularly encouraged.

University of Zurich

The Faculty of Science at the University of Zurich invites applications for an

Assistant Professorship in Inorganic Chemistry

We seek applications from excellent candidates in all areas of inorganic chemistry. To complement existing expertise in organometallic, biological and medicinal inorganic chemistry, we particularly welcome candidates from solid state chemistry, inorganic photochemistry or bioinorganic chemistry.

The successful candidate is expected to develop a strong, independent research program and to contribute to teaching. He or she will receive attractive startup funding and have access to the excellent infrastructure of the Department of Chemistry. Interactions with existing research groups are strongly encouraged. Detailed information on existing research areas of Inorganic Chemistry and of other related University Institutes can be found at http://www.mnf.unizh.ch. Zurich, with its two high level academic institutions offers both a stimulating environment for research with many opportunities for collaborations and a lively international atmosphere.

The position is a non-tenure track assistant professorship starting in summer 2007 or as soon as possible thereafter. Applications received by September 30th, 2006 will be guaranteed full consideration but we are open to exceptional candidates until the position is filled. Submissions should include a complete curriculum vitae, a short summary of past and future research interests, copies of three key publications and names and addresses of three potential referees and be sent to Prof. Dr. D. Wyler, Vice Dean of the Faculty of Science (MNF), University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. The application material should also be submitted in PDF-format to jobs@mnf.unizh.ch.

For inquiries please contact Prof. Dr. H. Berke, Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland, E-mail hberke@aci.unizh.ch. The University of Zurich is an equal opportunity employer. Applications from women are particularly encouraged.

Endowed Professorship Bioinformatics

Applications and/or nominations are invited for the **Endowed Professorship in Bioinformatics** at the College of Charleston. This is the first of two appointments to be made within the Center for Economic Excellence in Marine Genomics, a partnership between the College and the Medical University of South Carolina. It is anticipated that the appointment will be made at the level of Associate Professor or Professor in the Department of Biology at the College with a joint appointment at the Medical University. For information about the department, see **www.cofc.edu/~biology**.

The successful applicant will have a demonstrated track record as a collaborative scholar, a strong commitment to teaching and mentoring graduate and undergraduate students, and, ideally, will also have significant experience with the mechanisms for enhancing research value through economic development. Experience as a research team leader/program director is highly desirable. The successful candidate will provide academic and program leadership to the Bioinformatics Group within the Marine Genomics program in Charleston. This program focuses on applying genomic approaches to increasing understanding of the interactions of marine organisms with their environment, including infectious diseases, and the relationship between the oceans and human health. The Chairholder will lead an existing team of programmers and computational biologists and will drive the conceptual and theoretical interpretation of experimental results.

Historic Charleston SC, on the biologically diverse southeast Atlantic marsh, is a natural laboratory for an integrated effort to monitor, understand, protect and manage the marine environment. The Marine Genomics program currently comprises over 40 faculty, students and staff from the College, Medical University, SC Department of Natural Resources, National Oceanic and Atmospheric Administration and National Institute of Standards and Technology, as well as the Hollings Marine Laboratory (a partnership of the five Ft. Johnson organizations) on the Ft. Johnson marine campus, five miles from downtown Charleston. Information about the current Marine Genomics bioinformatics infrastructure can be found at: **http://marinegenomics.org** (see also BMC Genomics 2005,6:34).

More information about this position can be obtained at the School of Sciences and Mathematics website: **www.cofc.edu/~ssm** or from **Dr. Norine Noonan**, Dean, School of Sciences and Mathematics, at **NoonanN@cofc.edu** or the chair of the search committee, **Dr. Louis Burnett**, at **BurnettL@cofc.edu**. Applicants should send a statement of research interests and accomplishments, a *Curriculum vitae*, and the names and contact information for at least three references to: **Bioinformatics Chair, Office of the Dean, School of Sciences and Mathematics, College of Charleston, 66 George St., Charleston, SC 29424**. Nominations should be sent directly to the Dean. Applications and nominations will be held in confidence to the extent possible. Review of applications will begin on **August 28, 2006** and will continue until the position is filled.

FIOCRUZ

CENTER FOR TECHNOLOGICAL DEVELOPMENT IN HEALTH (CDTS)
OSWALDO CRUZ FOUNDATION (FIOCRUZ), Rio de Janeiro, Brazil

CAPES

VISITING-PROFESSOR, POST-DOCTORAL LONG-TERM COLLABORATION PROGRAM

The Oswaldo Cruz Foundation (FIOCRUZ; www.fiocruz.br), an institution affiliated to the Brazilian Ministry of Health, will this year start to build its Center for Technological Development in Health (CDTS in its Portuguese acronym), to be inaugurated in 2008. The CDTS 20,000 m² buildings will host technological platforms, animal experimentation facilities and flexible laboratories where FIOCRUZ will work in collaboration with public and private industrial sectors in the joint development of health products against neglected diseases and other diseases and conditions of epidemiological and/or economic importance to Brazil.

The CDTS project represents the second phase of FIOCRUZ's 2000-2007 strategic plan which strengthens the Foundation's activities in technological development of health products. The first phase implemented two internal R&D programs involving 80 R&D projects: Program for Technological Development of Health Products (PDTIS), related to vaccines, drugs, diagnostics, insecticides; Program for Technological Development of Public Health (PDTSP), related to the design and development of health policies and strategies. The CDTS will provide the necessary infrastructure and human resources to allow FIOCRUZ to fully benefit from the enabling environment provided by Brazil's 2004 Law on Innovation, which encourages partnerships between public and private sectors for the development and production of industrial goods.

In order to strengthen and mobilize the human resources needed for the CDTS, FIOCRUZ and CAPES (an agency of the Ministry of Education) will support up to 22 (twenty-two) Post-doctoral Fellowships (training young Brazilian scientists in public or private R&D centers of excellence) and up to 15 (fifteen) Visiting Professor Fellowships (supporting foreign professionals to collaborate with the CDTS project and participate in FIOCRUZ graduate programs).

An over-arching goal of the program will be to establish long-term institutional partnerships between FIOCRUZ and leading institutions. Skills are sought in the following areas:

R&D Areas

- Genomics
- Proteomics
- Bioinformatics
- Microarrays and nanotechnology
- Glycoprotein and lipid structure
- Protein crystallization

Technology Development

- Toxicology
- Production and purification of recombinant proteins and monoclonal antibodies
- Biological collections
- Animal experimentation
- Physical-chemical analysis

Technology Management

- Technology portfolio management
- Intellectual property rights
- Technology transfer
- Business plans
- Negotiations and agreements for public-private partnership

- The Post-doctoral program is open to Brazilian candidates who have completed their PhD/DSc work and would like to apply for post-doctoral training in the above areas in leading public or private institutions in Brazil or abroad and who on their return will be willing to join the CDTS project. Support will be for one year, renewable for an additional year.

- The Visiting Professor program is open to scientists and technology management professionals at PhD/DSc or equivalent level, and proven experience and outstanding achievement. Citizens from countries having diplomatic relationships with Brazil are eligible, from public or private sector institutions. Visits could be for short, medium and long stays for up to one year, renewable, working at FIOCRUZ. Stipends are available.

- Both programs will give priority to candidates who can demonstrate: (1) Strong support for their applications from the institution where the Post-doctoral stay will be performed or where the Visiting Professor is affiliated, including willingness to engage in long-term collaboration with FIOCRUZ after the training or visiting period; (2) Relevance of the training- or R&D activities to FIOCRUZ objectives and goals; (3) Previous experience pertinent to the CDTS project and its long-term goals. Candidates will be selected by a six-member expert panel nominated by FIOCRUZ and CAPES. The program will run until 31 December 2008.

For further information or to express interest, contact:
Center for Technological Development in Health (CDTS) - Oswaldo Cruz Foundation (FIOCRUZ) - Ministry of Health of Brazil
Casa Amarela, campus de Manguinhos - Av. Brasil 4365 - Rio de Janeiro, RJ 21040-900 - Brazil
e-mail: cdts@fiocruz.br :: Tel: +55-21-3885-1754 :: Fax: +55-21-2260-6707

CHAIR POSITION

Department of Pharmaceutical Sciences
Washington State University
Pullman, WA

The Department of Pharmaceutical Sciences invites applications from qualified candidates for a department chair position at the tenured full professor level to begin on or about September 1, 2007 (or negotiable). Candidates must have a Ph.D. and/or M.D. in a biomedical discipline and an active independently funded research program. Research in all areas related to pharmaceutical sciences will be considered, although cancer and toxicology will be given preference. The successful candidate will have leadership capabilities and a commitment to sustaining and expanding the research and teaching environment at Washington State University. Additional junior faculty positions are available for the successful candidate to further grow the department. Demonstrated competence in the development and/or implementation of professional and graduate curricula and an excellent record of mentoring is desirable. The position may include an endowed professorship and a future opportunity for an additional administrative role at the College-level. See department webpage (URL: **http://www.pharmacy.wsu.edu/PharmSci**) for additional information.

Screening of applicants will begin **September 1, 2006**. To apply, please submit the following: a letter of application explaining how the candidate's record relates to the qualifications and responsibilities listed; a curriculum vitae; and the names and contact information of three professional references only. Send (e-mail acceptable) to: **Raymond M. Quock, Ph.D., Search Committee Chair, Dept. of Pharmaceutical Sciences, College of Pharmacy, P.O. Box 646534, Washington State University, Pullman, WA 99164-6534; quockr@mail.wsu.edu**.

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Educator and Employer.

BIOENGINEERING
UNIVERSITY of CALIFORNIA, BERKELEY

FACULTY POSITION

The Department of Bioengineering in the College of Engineering at the University of California, Berkeley invites applications for a **TENURE-TRACK** or **TENURED** position at the assistant, associate, or full professor level in bioengineering. The Berkeley Department of Bioengineering enjoys a close relationship with the School of Medicine at the University of California, San Francisco (UCSF). Our interdisciplinary program offers outstanding opportunities for collaboration with distinguished researchers in related departments and colleges at Berkeley, UCSF, the Lawrence Berkeley National Laboratory, and within the greater Bay Area biotech community. This exceptional environment for teaching and research in a rapidly growing field will provide the successful candidate with a unique opportunity to provide intellectual and technological leadership in bioengineering.

We seek an individual with demonstrated excellence in the field to establish an active and innovative research program in **synthetic biology and/or cellular engineering**, including technology development and applications for engineering of macromolecules, pathways, cellular systems, and multicellular systems. Exceptional candidates in other areas of bioengineering will also be given consideration. Successful applicants will be expected to teach undergraduate and graduate courses in bioengineering and should have a strong commitment to and potential for excellence in teaching and leadership. To learn more about our department please visit **http://bioeng.berkeley.edu**. The level of appointment will be based on experience and qualifications.

To apply please email a curriculum vitae with a complete list of publications; a brief description of research accomplishments; a selection of publication reprints and teaching evaluations (if available); and a brief statement of research plans and teaching interests to: BioEsearch1119@berkeley.edu. Applicants should also arrange to have at least three letters of reference sent to the department directly. Potential reviewers are referred to the Statement of Confidentiality at http://apo.chance.berkeley.edu/evalltr.html. Inquiries and/or hard copy applications and reference letters should be addressed to: Professor Adam Arkin, Search Committee Chair, Department of Bioengineering, 459 Evans Hall MC 1762, University of California, Berkeley, CA 94720-1762. The review of applications will commence on **August 31, 2006** and will continue until the position is filled. The University of California is an equal opportunity affirmative action employer, committed to excellence through diversity.

CHAIR, DEPARTMENT OF MOLECULAR MEDICINE

School of Basic Biomedical Sciences

The School of Basic Biomedical Sciences within the College of Medicine at USF Health, seeks an outstanding scientist for the position of Chair of the Department of Molecular Medicine. USF Health has identified strategic research interests, which include Neurosciences, Cardiovascular, Allergy, Immunology & Infectious Diseases and Cancer Pathobiology and it is anticipated that the successful candidate will have research focused in one of these "signature" programs. USF is one of only 95 public and private universities in the U.S. that have been designated as Carnegie Comprehensive Doctoral Research University/Very High Research Activity.

Minimum requirements include a MD, PhD, or MD/PhD with a minimum of five years of experience as an Associate Professor or equivalent. A record of sustained accomplishments and evidence of leadership in his/her field, relevant administrative experience, and evidence of effective interpersonal, collaborative, and communication skills is required. The successful candidate is expected to have a distinguished record of scholarly activity, continuous NIH R01 and other extramural funding and requisite teaching experience in a medical/graduate curriculum. A legacy of building interdisciplinary programs and experience with successfully mentoring graduate and medical students, postdoctoral-fellows, and junior faculty is also required.

Applicants should submit, by email, a letter summarizing their qualifications and interests in the position, future research plans, curriculum vitae and the names and contact information of five references. Completed applications must be submitted to **Ms. Vanessa Ayer (vayer@health.usf.edu).** Competitive start-up packages and salaries will be provided commensurate with experience. Review of applications will begin July 10, 2006 and will continue until this position is filled. For more information, please visit this website: **http://hsc.usf.edu/medicine/dept_chair_announcement.html**.

USF Health is committed to increasing its diversity and will give individual consideration to qualified applicants for this position with experience in ethnically diverse settings, who possess varied language skills, or who have a record of research issues that support/benefit diverse communities or teaching a diverse student population. The University of South Florida is an Equal Opportunity / Affirmative Action / Equal Access Institution. For disability accommodations, contact Vanessa Ayer at 813-974-8349 within 5 days of an event. According to Florida law, search records, including applications and search committee meetings, are open to the public.

USF HEALTH UNIVERSITY OF SOUTH FLORIDA

50 YEARS

http://hsc.usf.edu · 12901 Bruce B. Downs Blvd, MDC 02 · Tampa, FL 33612

University of Idaho

Reproductive Biology, Faculty Position

The Department of Biological Sciences at the University of Idaho invites qualified individuals to apply for a tenure-track position in reproductive biology. Preference will be given to individuals at the assistant professor rank whose research interests focus on fish and complement current areas of strength in gamete physiology, developmental biology, evolutionary genetics, molecular biology of sex steroid receptors, gonad transplantation, and endocrine disruptor toxicology within the department (http://www.sci.uidaho.edu/biosci/). The applicant will be encouraged to conduct interdisciplinary research with faculty in the Center for Reproductive Biology (http://www.crb.wsu.edu), a joint initiative between the University of Idaho and nearby Washington State University. The University of Idaho has exceptional live fish holding facilities for both warm water (e.g. zebrafish) and cool water (e.g. trout) species maintained through the Aquaculture Research Institute (http://www.webs.uidaho.edu/aquaculture). The applicant will be expected to teach at both the undergraduate and graduate levels. A competitive salary and start-up package will be provided. A Ph.D. in Biology or a related field and postdoctoral experience is required.

For more information and to apply online, visit: http://www.hr.uidaho.edu. Send inquiries to biofac@uidaho.edu. Review of applications will begin on September 15, 2006; those received by that date will receive priority.

The University of Idaho is an affirmative action equal opportunity employer.

The Center for Cancer Immunology Research

Department of Immunology

The Department of Immunology at the University of Texas M. D. Anderson Cancer Center is undergoing a second-phase expansion. The department is part of the newly established Center for Cancer Immunology Research at M. D. Anderson, and is located in a new 132,000 sq. ft. research building that houses state-of-the-art flow cytometry, 2 photon imaging, transgenic and gene-targeting, protein expression, hybridoma, and histology facilities. Our existing faculty study cellular and molecular mechanisms of innate and adaptive immunity. Visit our Web site for more details at **www.mdanderson.org/departments/immunology**.

We invite applications for tenure track or tenured positions at all levels, with special considerations for those with established track records in high-level publications and peer-reviewed research funding. Although excellent investigators in all areas will be welcome, those working in the following areas are preferably invited to broaden our research basis: 1) immune and cytokine receptor signaling; 2) transcriptional, epigenetic, and small RNA regulation of immune development and responses; or 3) cell biology or systems biology analysis of immune cells. Competitive start-up packages will be provided to successful candidates. Houston, home of the Texas Medical Center with numerous exciting basic and clinical research opportunities, offers an affordable cosmopolitan living environment.

Interested individuals should contact:

Yong-Jun Liu, M.D., Ph.D.
Chair, Department of Immunology and
Director, Cancer Immunology Research Center
The University of Texas M. D. Anderson Cancer Center
South Campus Research Building
7455 Fannin, P.O. Box 301402
Unit 901
Houston, Texas 77030-1903

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®

M. D. Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at The University of Texas M. D. Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free environment.

MANAGER, TRANSLATIONAL ONCOLOGY FACILITY

As part of a strategic plan to expand efforts in the application of molecular tools to the conduct of clinical trials, Memorial Sloan-Kettering Cancer Center (MSKCC) invites applications for Manager of a new Translational Oncology Core Facility. We are looking for a dynamic scientist to assume day-to-day responsibility for a facility that will interface with existing MSKCC Core Facilities in Pathology, Genomics and Bioinformatics to conduct multi-parameter molecular analyses of clinical material. The manager will play a key role in developing a state-of-the-art laboratory-based facility that will coordinate the flow of samples through a pipeline that includes pathologically supervised tumor macro/micro dissection, DNA/RNA isolation and high throughput PCR-based amplification for a variety of genome-based assays. The Facility Manager will work closely with clinical and translational oncology researchers in the design and execution of projects involving molecular analysis of samples from patients enrolled on clinical trials and will play a critical role in evaluating emerging technologies for molecularly-based tissue analysis for potential incorporation into the new facility.

The successful candidate must have an M.D. or Ph.D. and hands-on experience with genome scale analysis of clinical material including nucleic acid isolation, PCR, instrumentation and automation, robotics, experimental design and bioinformatics scale data analysis. Prior experience in molecular oncology research using human tissue samples is highly recommended. At least two to three years of postgraduate training in academic or corporate research, attention to detail and accuracy, strong organizational and troubleshooting abilities, and outstanding communication skills are also required. Previous management and administrative experience are preferred.

Appointment will be made to the Laboratory (non-tenure) Track Faculty, equivalent to Assistant or Associate Research Professor at most universities. Salary and rank according to qualifications. Applications should be received by October 31, 2006.

FACULTY POSITIONS FOR PHYSICIAN-SCIENTISTS IN TRANSLATIONAL ONCOLOGY

The newly inaugurated Human Oncology and Pathogenesis Program (HOPP) at Memorial Sloan-Kettering Cancer Center (MSKCC) invites applications for tenure track faculty appointments at the level of Assistant, Associate or Full Member. HOPP will assemble outstanding physician-scientists across clinical disciplines in a single program to foster translational oncology research at the laboratory/clinical interface in an environment that encourages collaborative team science. Current research by HOPP faculty encompasses oncogenomic studies of various cancers, analysis of aberrant signal transduction pathways, preclinical evaluation of molecularly targeted agents and mechanisms of drug resistance. Successful candidates must demonstrate the ability to develop an independent research program as well as an interest in translational oncology. HOPP faculty will be housed in state-of-the-art laboratories in the new Zuckerman Research Center and jointly appointed in the Department of his/her appropriate clinical specialty at MSKCC. Faculty will also be eligible to hold appointments in the newly established Gerstner Sloan-Kettering Graduate School of Biomedical Sciences as well as the Weill Medical School and Graduate School of Medical Sciences of Cornell University.

MSKCC offers a unique and vibrant research environment with programs in Immunology, Pharmacology, Chemistry, Molecular Biology, Computational Biology, Genetics, Cell Biology, Developmental Biology, Cellular Biochemistry and Structural Biology and close links with the Rockefeller and Cornell communities. The presence of world-renowned clinical programs in cancer research, treatment and prevention offers unique opportunities for creative collaboration. Applicants must have an M.D. or M.D. Ph.D and postdoctoral experience. Applications should be received by November 30, 2006.

Please send cover letter, curriculum vitae, names of three references and a summary of current and proposed research programs (Faculty positions only) to: **Charles L. Sawyers, M.D., Chair, Human Oncology and Pathogenesis Program, c/o Carol Slattery, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021; E-mail: slatterc@mskcc.org.** Memorial Sloan-Kettering Cancer Center is an affirmative action, equal opportunity employer.

Memorial Sloan-Kettering Cancer Center
The Best Cancer Care. Anywhere.
www.mskcc.org

UCLA

Department of Ecology and Evolutionary Biology

Plant Ecology Faculty Position

The Department of Ecology and Evolutionary Biology at UCLA invites applications for an OPEN RANK, TENURE-TRACK, FACULTY POSITION in **Plant Ecology**, which is one of several anticipated hires in the next few years. The expected start date is September 2007. Candidate must have a Ph.D.; postdoctoral experience is desired. Salary is commensurate with education and experience. Successful candidates are expected to participate in both undergraduate and graduate teaching, to contribute to the intellectual activities of the department, and to maintain an externally funded research program. UCLA has outstanding academic support for faculty, including access to the UC Natural Reserve System, the Institute of the Environment, the Center for Tropical Research, the NSF Center for Embedded Networked Sensing (CENS), and the NSF Institute of Pure and Applied Mathematics (IPAM).

Interested applicants should submit a CV, statements of research and teaching interests, and names and addresses of three references online to **www.eeb.ucla.edu/plantecology**. Please use the following job number: **0830-0607-01** in all correspondence. Please contact Search Committee Chair **Phil Rundel** (**rundel@biology.ucla.edu**) for additional information. Reviews of applications will begin **September 30, 2006**.

UCLA is an Equal Opportunity/Affirmative Action Employer. The Department has a strong commitment to the achievement of excellence and diversity among its faculty and staff.

Bauer Center for Genomics Research

Bauer Center for Genomics Research Harvard University Fellow Positions in Genomics or Systems Biology

Harvard University's Bauer Center for Genomics Research seeks outstanding applicants to become Fellows in the center.

The Bauer Center is an intensively collaborative, interdisciplinary center, where scientists from a wide variety of backgrounds study cellular pathways and networks, with the goal of finding general principles underlying the structure, behavior and evolution of cells and organisms. The Bauer Fellows are independent researchers who receive funding for a group of up to three people. Fellows are appointed for a three-year term, with the expectation that it will be extended to five years.

For more information about the Bauer Center and the Fellows program, visit our website at **http://www.cgr.harvard.edu**. For application procedures, see **http://cgr.harvard.edu/fellows/fellows_program.html**.

Applications are welcome at any time, but only those received by **15 September 2006** are guaranteed full consideration.

Harvard University is an Affirmative Action, Equal Opportunity Employer.

WILLIAM & MARY
VIMS
VIRGINIA INSTITUTE OF MARINE SCIENCE
SCHOOL OF MARINE SCIENCE

Seawater Laboratory Director

The new Seawater Research Laboratory, scheduled for completion in Spring, 2007 at the Virginia Institute of Marine Science (VIMS), will feature state-of-the-art wet lab space for all aspects of interdisciplinary research including a large aquatic animal holding and quarantine laboratory, aquatic animal disease challenge laboratories, and aquatic toxicology laboratories. The new water polishing system can provide 900 gallons per minute of seawater to support cutting-edge research, along with flexible-use laboratories for ecological and physical sciences investigations and dry general use preparatory and analytical laboratories.

VIMS is searching for an individual with the vision to serve as Director of the new facility and develop this new marine research laboratory to its full research and education potential. Extensive experience in wet laboratory management, wet lab physical plant operations and maintenance and aquatic animal holding and exposure systems design and construction is ideal. Duties include overseeing all aspects of daily operation, assuring compliance with environmental discharge requirements, aiding all aspects of experimental systems design and construction, and assisting the promotion, development, staffing and funding of the new facility.

Qualifications: Advanced degree in a sub-discipline of Marine Science or Marine Engineering preferred along with experience in wet research laboratory management, operation and maintenance, including trouble shooting of water delivery, water polishing and waste disposal machinery. Familiarity with design, construction and operation of flow-through and closed recirculating seawater systems, along with water chemistry and water quality maintenance procedures. Knowledge regarding marine animal husbandry practices is highly desirable along with ability to work diplomatically with all constituents and tight research schedules. Experience in the development of self-sustaining, intramural and extramural research support services is highly beneficial. For a complete job description along with details about the new State-of-the-Art Seawater Research Laboratory at the Virginia Institute of Marine Science visit **http://www.vims.edu/srl/**

This position will be at the level of Professional Faculty (non-tenure track), and salary will be commensurate with expertise and experience. Application materials (C.V. and contact information for three references) may be submitted by mail or electronically (http://www.vims.edu/srl/). Mail applications to: **Ms. Linda Caporale, Virginia Institute of Marine Science, P.O. Box 1346, Gloucester Point, VA 23062**.

Review begins **August 24th**, and will continue until an appointment is made.

William and Mary is an AA/EEO Employer. Underrepresented groups are strongly encouraged to apply.

NIH-Postdoctoral Positions

New York Medical College is one of the nation's largest private health sciences universities, leading the way in improving the health of the population through the education of physicians, scientists and health providers. It is situated in the picturesque Hudson Valley region in Westchester County, just 30 minutes north of New York City. We currently have 3 NIH Postdoctoral Positions.

Two NIH-funded postdoctoral positions are available to study the role of EC-SOD, HO-1 genes (using pharmacological and genetic probes) and mimetic peptide in vascular system (Circulation 2005 vol. 111 pages 3126-3134). Positions are available immediately and applications will be accepted until the positions are filled. Salary will be commensurate with experience and positions can be 1-4 years. Applicants should a have PhD and significant experience with standard culture techniques /molecular probes, animal experience is a plus. Successful candidates will be encouraged to develop their own approaches and strategies and be able to interact with other individuals in the laboratory.

One NIH-position is for a postdoctoral fellow experienced in retroviruses, gene transfer and stem cells. Applicants should have PhD or equivalent, the successful candidate will be encouraged to develop their own approaches and strategies.

Please send a letter of interest, CV and three letters of recommendation, one of the letters is from your current mentor to:

NEW YORK MEDICAL COLLEGE CHARTERED 1860

Dr. Nader G Abraham,
Professor Of Pharmacology
New York Medical College
Valhalla, NY 10595
E-mail: **Nader_Abraham@NYMC.EDU**

FACULTY POSITIONS IN CELLULAR AND MOLECULAR IMMUNOLOGY

The Immunology Program at the H. Lee Moffitt Cancer Center & Research Institute and the University of South Florida's College of Medicine, Department of Interdisciplinary Oncology, are seeking highly qualified (Ph.D. or M.D.) applicants for tenure track positions in Cellular and Molecular Immunology at Assistant, Associate and Full Professor levels. While applicants in all areas of Cellular and Molecular Immunology may apply, we are especially interested in individuals with expertise in T cell and dendritic cell biology, innate immunity, and tumor immunology. Applicants with a strong interest in bridging basic and translational research in the area of cancer immunology and immunotherapy are especially encouraged to apply. Successful applicants will be expected to develop an outstanding research program in their area of interest.

The Assistant Professor must have at least four years of postdoctoral experience in tumor immunology and high quality publications in peer-reviewed journals. The Associate/Full Professor must have a proven track record of independent research and demonstrated sustained extramural funding. In addition, the Associate Professor rank requires at least five years of experience with continuing and productive service as an Assistant Professor. The Professor rank requires documentation of national recognition, leadership ability and at least five years of experience with continuing and productive service as an Associate Professor. Salary is negotiable.

The Moffitt Cancer Center and Research Institute provides an exceptional environment for basic and translational research in Immunology, Molecular Oncology and Drug Discoveries. Extensive state-of-the-art core facilities are available for flow cytometry, gene profiling, proteomics, mouse model development and drug discovery. Successful applicants will be provided generous laboratory and office space in the new Vincent A. Stabile Research Building.

Please reference position DIO0526. Send curriculum vitae and a brief statement of major academic interests in one single pdf document to The Immunology Search Committee at koransky@moffitt.usf.edu. Application review begins September 1, 2006. Applications will be accepted and continuously reviewed until the position is filled.

USF Health is committed to increasing its diversity and will give individual consideration to qualified applicants for this position with experience in ethnically diverse settings, who possess varied language skills, or who have a record of research that supports/benefits diverse communities or teaching a diverse student population.

H. LEE MOFFITT Cancer Center & Research Institute

The End Of Cancer Begins Here.

A National Cancer Institute Comprehensive Cancer Center At the University of South Florida

USF University of South Florida College of Medicine

The University of South Florida is an EO/AA/EA institution. For disability accommodations, contact Kathy Jordan (813-632-1451) a minimum of five working days in advance. According to Florida law, search records, including applications and search committee meetings, are open to the public.

www.moffitt.usf.edu

GNS SCIENCE TE PŪ AO

GNS Science, Te Pū Ao, is a Crown Research Institute.Our core purpose is to understand earth systems and physics-based technologies and to transform this knowledge into economic and social benefits for New Zealand.

Quaternary Paleoclimate Scientist

Scientist/Senior Scientist

We are seeking an experienced marine or terrestrial geoscientist to run a paleoclimate project, with emphasis on the last million years. The appointee will develop an integrated New Zealand Quaternary history of climate and landscape processes in the context of global change and integrate results into international paleoclimate research programmes, and local databases.

To be successful you will have specialist skills in one or more fields relevant to paleoclimate research and have already developed international networks and collaborations in that area. The ability to develop new or improved research techniques and procedures would be an advantage.

If you are a team player with a 'can do' attitude, have an interest in Earth Sciences and want to work for a dynamic, progressive, multi-cultural organisation then GNS could be the place for you.

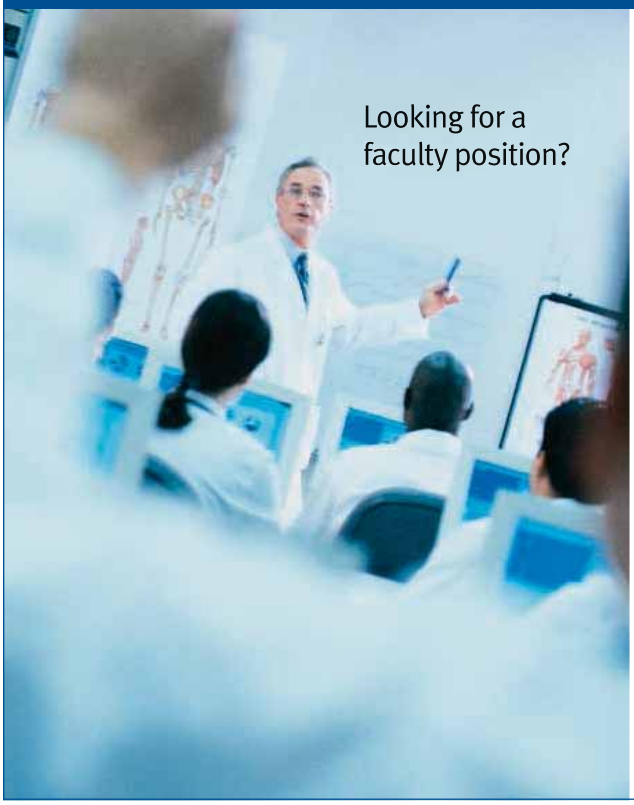
Further information can be obtained from our website or by phoning Andrea McLiver. Please send a covering letter, CV and completed application form to Human Resources or email us at careers@gns.cri.nz

Applications close on 22 September 2006.

**GNS Science, PO Box 30 368,
Lower Hutt, New Zealand
T 64-4-570 1444, F 64-4-570 4748,
www.gns.cri.nz/careers**

Faculty Careers 2

A *Science* Advertising Supplement



Be sure to read this special ad supplement devoted to faculty career opportunities in the **8 September issue of *Science*.**

Find faculty position listings and other career resources online at **www.sciencecareers.org.**

For advertising information, contact:

U.S. Daryl Anderson
phone: 202-326-6543
e-mail: danderso@aaas.org

Europe and International
Tracy Holmes
phone: +44 (0) 1223 326 500
e-mail: ads@science-int.co.uk

Japan Jason Hannaford
phone: +81 (0) 52 789-1860
e-mail: jhannaford@sciencemag.jp

ScienceCareers.org
We know science AAAS

Assistant/Associate Professor of Physiology
Southern Illinois University

The Department of Physiology at Southern Illinois University Carbondale's School of Medicine, Carbondale, invites applications for a **tenure-track faculty** position at the rank of Assistant or Associate Professor. Preference will be for an investigator in cancer biology, however those who complement existing strengths in neuroscience and reproductive endocrinology are also encouraged to apply. The successful candidate will be expected to contribute to the medical school, graduate and/or undergraduate teaching responsibilities of the Department. The position includes a 100% time **12-month state-funded competitive salary**, spacious research facilities, and substantial start-up funds. The University has over 22,000 students making it the second largest public university in Illinois, and is located 2 hours southeast of St. Louis, MO on the border of the scenic Shawnee National Forest. The Department has established graduate and undergraduate research programs in molecular, cellular, and systemic physiology. Applicants must have a Ph.D. (or equivalent degree) and evidence of high quality research potential. Postdoctoral experience is required. For appointment at the associate professor level, the candidate should currently have faculty status at a university (or equivalent), an extramurally funded research program, and a strong record of research productivity. This is a security sensitive position. Before any offer of employment is made the University will conduct a pre employment background investigation, which includes a criminal background check. Additional Departmental information can be obtained via the following Web address: **http://www.siumed.edu/physiology**/.

Review of applications will begin October 1, 2006 and will continue until the position is filled. Applicants are asked to submit curriculum vitae, description of research and teaching interests, and arrange to have at least three letters of reference sent to: **Faculty Search Committee, c/o Dr. Michael Collard, Department of Physiology, School of Medicine, Mail Code 6523, Southern Illinois University Carbondale, 1135 Lincoln Drive, Carbondale, IL 62901.**

SIUC is an Affirmative Action/Equal Opportunity Employer that strives to enhance its ability to develop a diverse faculty and staff to increase its potential to serve a diverse student population. All applications are welcomed and encouraged, and will receive consideration.

POSITION ANNOUNCEMENT
DEPARTMENT OF POULTRY SCIENCE
THE UNIVERSITY OF GEORGIA
ATHENS, GA

Molecular Biologist
Assistant Professor, Associate Professor or Professor

POSITION: This is a 12-month tenure-track faculty position with an appointment in research (75%) and instruction (25%). The individual selected for this position will be expected to develop a contemporary research program in molecular biology that will be supported, in part, by extramural funding. Potential research focuses could include, but are not limited to, endocrinology, genetics, nutrition, physiology and reproduction. Teaching responsibilities may include departmental undergraduate and graduate courses in molecular/cell biology, as well as in the area of the candidate's expertise. The successful applicant will also be expected to develop a strong graduate training program. Additional duties include undergraduate advisement, student recruitment and service to the poultry industry. The University of Georgia is a preeminent Land Grant university with excellent opportunities for collaboration with faculty that have diverse research interests. Furthermore, there are excellent core facilities, including Functional Genomic and Proteomic centers, Sequencing and Synthesis facilities, as well as Ultrastructural and Flow Cytometric facilities that are available for molecular biology research.

QUALIFICATIONS: Qualified applicants should possess a PhD in Molecular Biology, Poultry Science, Animal Science or a related field, as well as postdoctoral training for candidates applying at the assistant level. Candidates at the associate and professor level should have a record of successful grantsmanship and teaching excellence. Applicants with training and experience with non-avian animal models are encouraged to apply. The ability to integrate molecular biology concepts into multidisciplinary research programs in poultry is essential.

APPLICATIONS: Applicants should submit a letter of application that addresses teaching interests and a detailed description of their proposed research program, a curriculum vitae and official transcripts. Three letters of reference should be forwarded to the address below. Applications received by **November 1, 2006** are assured full consideration. Position available January 1, 2007. Send applications to: **Dr. Michael P. Lacy, Department of Poultry Science, The University of Georgia, Athens, GA 30602-2772; Telephone: (706) 542-1351; Fax: (706) 542-1827; E-mail: mlacy@uga.edu**.

AWARDS

JDRF Juvenile Diabetes Research Foundation International

dedicated to finding a cure

Scholar Award

The Juvenile Diabetes Research Foundation (JDRF) announces a Scholar Award to provide sustained support to scientists to perform pioneering basic or clinical research aimed at accelerating the progress toward a cure of type 1 diabetes and its complications. JDRF desires to support scientists with exceptional talent, vision, and creativity who are willing to take risks and attempt new research approaches.

JDRF Scholar Awards: Up to five years of funding of $250,000 annually and up to four awards will be granted in 2007. Eligible investigators must hold an academic degree in a scientific discipline, hold an independent research position at a university, health-science center, or comparable institution, and have ten years of relevant research experience.

Deadline: Applications must be received by JDRF no later than **September 18, 2006**.

Program and application details and JDRF contact information: **http://www.jdrf.org/JDRFscholar**

TU/e technische universiteit eindhoven

Full professor Photonic Nano-materials

The department of Applied Physics of the Eindhoven University of Technology (TU/e) invites candidates to apply for this new chair in the group Photonics and Semiconductor Nanophysics. Candidates should have an excellent international reputation in this research area and a strong interest in multi-disciplinary research. Information on the department of Applied Physics and a chair profile can be found at **http://www.phys.tue.nl** and at **http://www.phys.tue.nl/psn**

The TU/e - a highly competitive university of technology - is located in Eindhoven in The Netherlands, in a European Technology Hotspot with high-tech companies such as Philips and ASM-Lithography and many start-ups.

UNITED STATES DEPARTMENT OF AGRICULTURE

Cooperative State Research, Education, and Extension Service (CSREES)

ASSOCIATE ADMINISTRATOR

The Department of Agriculture (USDA) is seeking to fill the position of CSREES Associate Administrator. As CSREES Associate Administrator, the incumbent participates fully with the Administrator in all aspects of CSREES programs and policies. The incumbent shares responsibilities in working with partners and customers to advance research, extension, and education in the food and agricultural sciences and related environmental and human sciences to benefit people, communities, and the Nation. Programs under the direction of the Administrator and Associate Administrator are financed by approximately $1.2 billion in Federal funds and accomplished through the efforts of approximately 450 CSREES employees. The incumbent has frequent contacts with top officials of USDA, other government agencies, cooperative extension services, state agricultural experiment stations, colleges and universities, private organizations and corporations, national and international institutions, Departments and Ministries of Agriculture in other nations, and members of Congress and their staffs.

This is a Senior Executive Service position. The salary ranges from $109,808 to $165,200, commensurate with experience. Applicants must meet mandatory qualifications, as specified in vacancy announcement **CSREES-SES:06-02**, and address specific executive core and technical qualifications. For more information on the position, call **Betty Lou Gilliland** on **202-720-5506**. For information on the application process, call **Deborah Crump** on **301-504-1448**. A copy of the vacancy announcement may be located on the Office of Personnel Management web page at **http://www.usajobs.opm.gov/**. Applications must be received by **September 26, 2006**.

U.S. CITIZENSHIP REQUIRED
USDA IS AN EQUAL OPPORTUNITY PROVIDER AND EMPLOYER

SEARCH EXTENDED

Assistant Professor of Plant Ecological Genomics
School of Integrative Biology
University of Illinois at Urbana-Champaign

The School of Integrative Biology and the Department of Plant Biology seek an outstanding early career scientist with a background in interdisciplinary research involving aspects of plant ecology, ecosystem biology, plant environmental physiology, genomics, and statistics and/or bioinformatics for a nine-month, tenure-track faculty position at the assistant professor level. Candidates must have a Ph.D. The ideal candidate will have extensive familiarity with plant ecology, the ability to develop and implement statistical protocols for complex data analysis, and experience with appropriate genomic and informatic tools to address ecosystem level issues. This new faculty member is expected to develop an externally funded research program to investigate environmentally sensitive genes and processes that shape ecological interactions. The School has a particular interest in interactive responses of plants to abiotic stresses with anthropogenic changes and has world-class facilities for research in this area.

The successful candidate will have the opportunity to be part of a dynamic and well-established life science faculty, as well as a broadly based genomics community forming around the Institute for Genomic Biology, housed in a new state-of-the-art facility. Teaching obligations include participation in appropriate graduate and undergraduate instruction, including introductory level biology as well as upper level offerings in ecological genomics and related topics. The proposed starting date is January 2007, or negotiable after closing date; salary is commensurate with experience.

To ensure full consideration, applicants must submit a CV and statements of research and teaching interests and arrange for three letters of reference to be sent no later than **September 15, 2006**. Applicants may be interviewed before the closing date; however, no hiring decision will be made until after the closing date. Please send materials to the **Ecological Genomics Search Committee, School of Integrative Biology, University of Illinois, 286 Morrill Hall, 505 South Goodwin Ave., Urbana, IL 61801 (phone: 217/ 333-3044; fax: 217/ 244-1224; email: sib@life.uiuc.edu)**.

The University of Illinois is an Affirmative Action, Equal Opportunity Employer.

POSITIONS OPEN

ASSISTANT PROFESSOR POSITIONS
Biochemistry and Inorganic Chemistry

The Department of Chemistry and Biochemistry, California State University, Northridge, invites applications for Tenure-Track positions in biochemistry and inorganic chemistry, effective August 20, 2007. The successful candidate will have primary teaching responsibilities in undergraduate and graduate biochemistry or inorganic courses, and will be expected to develop a research program involving undergraduate and M.S. students. In biochemistry, preference will be given to candidates with postdoctoral experience and a strong background in chemistry. Review of applications will begin September 22, 2006, and continue until the position is filled. Submit applications to: **Chair, Biochemistry Hiring Committee.** The area of specialization in inorganic chemistry is open. Postdoctoral work and experience using x-ray diffractometers and solving crystal structures is desirable. Review of applications will begin October 1, 2006, and continue until the position is filled. Submit applications to: **Chair, Inorganic Hiring Committee.** Send curriculum vitae, a detailed description of research plans, a statement of teaching philosophy, and have three letters of recommendation sent directly by references to: **Department of Chemistry and Biochemistry, California State University, Northridge, CA 91330-8262; e-mail: taeboem.oh@csun.edu.** *California State University, Northridge is an Equal Opportunity Employer committed to excellence through diversity.*

TENURE-TRACK ASSISTANT/ASSOCIATE PROFESSOR

The Department of Environmental and Occupational Health, School of Public Health, University of Medicine and Dentistry of New Jersey (UMDNJ), invites applications for a tenure-track Assistant/Associate Professorship. Teach and mentor graduate students. Develop and sustain a vigorous, extramurally funded research program focused on the development and application of novel biomarkers for exposure, health effects, and/or susceptibility. Academic experience commensurate with the level of the appointment, and a demonstrated record of publications and extramural grants a must. An attractive package will be available, including relocation cost reimbursement, state-of-the-science laboratory space, and startup funds.

Complete applications received by October 1, 2006, will be assured of consideration. The earliest start date is approximately January 15, 2007. Send curriculum vitae, statement of research and teaching goals, and contact information for three references to: **Ms. Irene Karmazsin, Faculty Search Committee, University of Medicine and Dentistry of New Jersey-School of Public Health, 683 Hoes Lane W., Room 127, Piscataway, NJ 08854, e-mail: karmazif@umdnj.edu.** *Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

POSTDOCTORAL SCIENTIST

We are seeking an individual to carry out research in biodiversity of microbes and small eukaryotes at the molecular level in soil and marine sediments. The successful applicant will have a Ph.D. in a relevant biological science, experience with extracting DNA from soil, PCR amplification, high throughput sequencing and phylogenetic analysis of large datasets. Send curriculum vitae with three references and reprints to: **Dr. James Garey, Division of Cell Biology, Microbiology and Molecular Biology, University of South Florida, 4202 E. Fowler Avenue SCA110, Tampa, FL 33620,** or via **e-mail: garey@cas.usf.edu.**

The University of South Florida is an Equal Opportunity, Affirmative Action, Equal Access institution. For disability accommodations, please contact ***Ms. Dawn McGowan*** *at* ***telephone: 813–974–8088*** *at least five working days in advance. According to Florida law, applications and meetings regarding them are open to the public.*

POSITIONS OPEN

DEPARTMENT CHAIR

The Department of Biochemistry and Molecular Biology at Colorado State University invites applications for a new Chair. The successful candidate will have an internationally recognized research program complementing one or more existing strengths of the Department in cellular biochemistry, structural biology, and eukaryotic gene expression. The University has initiatives in infectious disease and genomics/proteomics that might be of interest to candidates. The Chair will provide vision and dynamic leadership in guiding the Department's research, teaching, and service missions. Required credentials include a Ph.D. degree in biochemistry or related field with research, teaching, and service experience commensurate with an appointment as a tenured Professor. Further information can be found at **website: http://www.bmb.colostate.edu/index.cfm.**

To apply, submit a cover letter, curriculum vitae, and statements of research and service experience, departmental leadership philosophy, and vision for undergraduate/graduate education to **website: http://www.natsci.colostate.edu/searches/biochem/.** Applicants should provide names and contact information online for three references as soon as possible to allow references time to write letters. Referees will receive instructions by e-mail for submitting letters online, or may mail them to: **Biochemistry and Molecular Biology Chair Search, c/o Department of Biology, 1878 Campus Delivery, Colorado State University, Fort Collins, CO 80523-1878.** For full consideration, applications should be received by September 15, 2006, although applications will be considered until the position is filled. *Colorado State University is an Equal Employment Opportunity/Affirmative Action Employer.*

BIOLOGY, ASSISTANT PROFESSOR

The Department of Biology at Shippensburg University invites applications for a tenure-track **VERTEBRATE PHYSIOLOGIST** position starting August 2007. Responsibilities include instruction of the following: a junior/senior level animal physiology course, an upper division undergraduate/graduate course in the area of candidate's specialty, and introductory courses for majors and nonmajors. The successful candidate will be expected to have a Ph.D. from an accredited institution completed by December 31, 2006. A successful demonstration of teaching effectiveness, a scholarly seminar, and evidence of a commitment to understanding diverse populations will be required as part of the on-campus interview. Applicants should send curriculum vitae, copies of transcripts (both graduate and undergraduate), a brief statement of teaching philosophy and research interests, plus the names, addresses, and telephone numbers of three references to: **Biology Search Committee, 1871 Old Main Drive, Shippensburg, PA 17257.** Review of application materials will begin on November 15, 2006, and will continue until the position is filled. *All applicants must furnish proof of eligibility to work in the United States upon appointment. Shippensburg University is an Equal Opportunity Employer.*

STANFORD UNIVERSITY
Department of Chemistry

TWO FACULTY POSITIONS, one at any level, in organic chemistry, and one at the junior level, in biological or physical chemistry. Completed applications must be received by October 2, 2006. Appointment commences on or after September 1, 2007. Applicants must be strongly motivated toward creative research and committed to undergraduate and graduate teaching. All applications should include: current curriculum vitae and publications list; brief statement of research interests; and three letters sent on your behalf directly to: **2006-2007 Organic Chemistry Search Committee,** or to: **2006-2007 Biological or Physical Chemistry Search Committee, Department of Chemistry, Stanford University, Stanford, CA 94305-5080.** *Stanford University is an Equal Opportunity, Affirmative Action Employer.*

POSITIONS OPEN

FACULTY POSITION IN CELL BIOLOGY
University of Texas Southwestern Medical Center at Dallas

The Department of Cell Biology of the University of Texas Southwestern Medical Center at Dallas seeks applications for a tenure-track position at the level of **ASSISTANT PROFESSOR.** Applicants must have a Ph.D. or M.D. degree, at least two years of postdoctoral training, and experience teaching human anatomy. Candidates are expected to develop an independent research program. Preference will be given to candidates with research programs in one of the following general research areas: cell biology, neuroscience, or functional morphology. The successful candidate will be joining a highly interactive and collegial teaching faculty.

Applicants should e-mail their curriculum vitae, the names of three references, and a brief description of their research goals to the attention of **Dr. Richard Anderson** at **e-mail: cb.recruitment@utsouthwestern.edu.**

For more information go to **website: http://www.swmed.edu/home_pages/cellbio/dw/index.html.** *The University of Texas Southwestern Medical Center is an Equal Opportunity/Affirmative Action Employer.*

FACULTY POSITION IN CELL AND DEVELOPMENTAL BIOLOGY

The Department of Cell and Developmental Biology at Oregon Health and Science University invites applications for a full-time tenure-track or tenured appointment at the level of **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR.** We seek applicants who use zebrafish as a model organism to study fundamental problems. Research interests of the current faculty members include developmental biology, cell biology, intracellular protein dynamics, cell-cell signaling, and cancer biology. Applicants at the Associate or Full Professor level should have an established research program with extramural funding. Please submit applications including curriculum vitae, a statement of research interests, and the names, addresses, and telephone numbers of three references to: **Faculty Search Committee, Department of Cell and Developmental Biology, L215, 3181 S.W. Sam Jackson Park Road, Portland, OR 97239-3098.** Screening of applications will begin September 15, 2006, and applications will be considered until the position is filled. *Oregon Health and Science University is an Equal Opportunity/Affirmative Action Employer.*

RESEARCH SCIENTIST

Stony Brook University seeks applications for a Research Scientist position in cancer prevention. Required: doctoral degree or foreign equivalent in a related field with three years of experience with molecular biology, and immunological and biochemical laboratory procedures, of which one year must be postdoctoral. Must have experience with cell culture and record of publications. Candidate must be able to work under limited supervision and communicate effectively. Preferred: evidence of independent investigation along with additional postdoctoral training. Send cover letter and resume to: **Dr. Basil Rigas, Cancer Prevention Laboratory, Life Sciences Building, Room 06, Stony Brook University, Stony Brook, NY 11794-5200,** or **fax: 631-632-1992.** For more information or to apply online visit **website:http://www.stonybrook.edu/cjo.** *Equal Opportunity/Affirmative Action Employer.*

The North Carolina State University Bioinformatics Research Center invites applications for a tenure-track **ASSISTANT PROFESSOR** with research emphasis in one or more of the following areas: biological networks, genomics, proteomics, and statistical genetics. All applicants must have a Ph.D. in statistics or a related field. Postdoctoral research experience is preferred. For further details and instructions on how to apply, please visit **website: http://bioinformatics.ncsu.edu.** *Affirmative Action/Equal Opportunity Employer.*

SYMPOSIA

AWARDS

POSITIONS OPEN

IMMUNOLOGY ASSISTANT PROFESSOR
at the University of Minnesota

The Department of Laboratory Medicine and Pathology in the University of Minnesota Medical School, in collaboration with the Center for Immunology, is seeking to hire a Tenure-Track faculty candidate.

Candidates should have experience, interest, and demonstrated productivity in basic aspects of innate or adaptive immunity, using either human or animal models. Candidates must hold a Ph.D., M.D., or equivalent degrees and have at least three years of relevant postdoctoral experience. The successful candidate will be expected to develop an independent and innovative externally funded research program, and will have the opportunity to participate in the teaching mission of the university. The goal is to broaden the range of expertise and research programs within the Center for Immunology.

Interested candidates can learn more about faculty interests, facilities, and educational programs at websites for the Center **(website: http://www.immunology.umn.edu/),** the Department **(website: http://pathology.umn.edu/)** and the Microbiology, Immunology, and Cancer Biology graduate program **(website: http://www.micab.umn.edu/).**

Applicants should attach their curriculum vitae, a statement of research interests, and the names and contact information for three references online at **website: http://www1.umn.edu/ohr/employment/index.html,** job requisition number 140834, or cut and paste the following into your web browser: **employment.umn.edu/applicants/Central?quickFind=53692.**

BIOMEDICAL SCIENCE and EDUCATION MANAGER
Pittsburgh Supercomputing Center
Carnegie Mellon University

Pittsburgh Supercomputing Center (PSC), a recognized leader in engineering and computer science, offers a challenging career opportunity as Biomedical Science and Education Manager (BSEM). The BSEM assists with the scientific and strategic direction of the National Resource for Biomedical Supercomputing (NRBSC), coordinates training, represents the NRBSC in forums, has responsibility for reporting departmental findings, developing new projects and assists in securing funding.

Minimum qualifications: Ph.D. relevant to computational biomedicine. Four years of management/supervisory experience; computational research experience including a commensurate publication record. Broad knowledge of biomedical research. Ability to network, recognize opportunities, and develop collaborations. Strong managerial skills including ability to manage multiple concurrent projects, set priorities and motivate individuals. Strong problem-solving and interpersonal skills. Excellent oral and written communication skills.

For additional information and to apply online go to **website: http://hr.web.cmu.edu/.** Click on careers at Carnegie Mellon and enter position 2479.

Carnegie Mellon University is an Affirmative Action/Equal Opportunity Employer committed to diversity.

ADMINISTRATIVE MANAGER/ SCIENCE WRITER

Staff scientist responsible for managing a large, multi-institutional HIV vaccine research effort funded by the Gates Foundation. Individual will be responsible for coordinating programmatic and operational aspects of research projects through interactions with internal and external academic scientists, pharmaceutical investigators, and foundation representatives. Position will also require working with laboratory scientists to manage and present data, and write manuscripts.

Requirements: Ph.D. in a life science, with strong interest in immunology, virology, and molecular biology; exceptional writing and organizational skills.

Please send cover letter and resume to: **Ms. Nicole Siciliano** at **e-mail: nsicilia@bidmc.harvard.edu.**

POSTDOCTORAL FELLOWSHIPS
The Center for Lung and Vascular Biology
University of Illinois at Chicago

The Center for Lung and Vascular Biology at University of Illinois at Chicago announces the availability of Postdoctoral Training Fellowships sponsored by the National Institutes of Health-funded Training Program in Lung Biology and Pathobiology (**website: http://www.uic.edu/depts/mcph/lung%20biology_T32.htm).** Trainees will focus their research efforts on vascular biology and lung injury and repair, the cellular and humoral basis of lung injury and cell signaling, and the regulation of lung function. Trainees will gain experience in an environment that fosters independent and creative thinking in a collegial atmosphere. *Applicants must be citizens or permanent residents of the United States* and have completed requirements for a doctoral degree. The ideal candidate should have experience in lung biology and physiology, vascular biology, or cell biology, and as well as excellent communication skills. For the fullest consideration please submit a letter of interest, curriculum vitae, copies of recent publications, and contact information for three references to:

Dr. Asrar B. Malik
Attn: Dr. Kelly Price, Ph.D.
Department of Pharmacology (MC 868)
University of Illinois at Chicago
835 S. Wolcott Avenue E403
Chicago, IL 60612
E-mail: lkp@uic.edu

The University of Illinois at Chicago is an Equal Opportunity/Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

BOTANIST

The Oberlin College Biology Department invites applications for a full-time, tenure-track faculty position beginning fall semester of 2007-2008. We seek an individual broadly trained in plant evolution and systematics. Initial appointment is for four years at **ASSISTANT PROFESSOR** or higher. Requirements: Ph.D. in hand by fall 2007; demonstrated interest and previous or potential excellence in undergraduate teaching. Postdoctoral experience strongly desired. Submit statements of teaching and research interests, curriculum vitae, official undergraduate and graduate transcripts, and three letters of reference to: **Roger Laushman, Chair, Biology Department, Oberlin College, Oberlin, OH 44074,** by September 15, 2006. Review of applications may continue until position is filled. Direct questions to **e-mail: roger.laushman@oberlin.edu.** *Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION
Center for Infectious Medicine
Department of Medicine
Karolinska Institutet, Sweden

Position available to study human stromal cell function and dendritic cell development in steady state and chronic inflammatory diseases. Work involves 3D imaging of cell-cell interactions in extracellular matrices and tissue explants. Previous experience in mesenchymal/stromal cell differentiation and function is desirable. *Priority may be given to applicants within the European Union.* Please send your curriculum vitae and contact details of three references to **Mattias Svensson** at **e-mail: mattias.svensson@ki.se.**

NIH-funded **POSTDOCTORAL POSITION** is available immediately at Florida International University (FIU) Department of Chemistry and Biochemistry to study transcription-coupled DNA supercoiling. A Ph.D. is required. Strong background in biochemistry, molecular biology, or microbiology is preferred. If interested, please send your curriculum vitae and three reference letters to **Dr. Fenfei Leng** at **e-mail: lengf@fiu.edu. Website: http://www.fiu.edu/~lengf.** *FIU is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

RESEARCH ASSISTANT PROFESSOR and **POSTDOCTORAL POSITIONS** available immediately in **Dr. Wang's** laboratory **(website: http://www.usd.edu/med/biomed/faculty/mentors.cfm)** in the Division of Basic Biomedical Sciences, Sanford School of Medicine at the University of South Dakota, Vermillion, South Dakota. Highly self-motivated recent graduates of Ph.D., M.D., or M.D./Ph.D. with the desire for training in cardiac biology are invited to apply for the Postdoctoral positions. Research Assistant Professor requires a doctoral degree in biomedical sciences (Ph.D., M.D., M.D./Ph.D., or equivalence) and a minimum of two years of postdoctoral training with proven skills in scientific writing and daily supervision of trainees in a cardiac biology laboratory. Prior research experience in murine physiology, molecular biology and genetics, morphology, genomics, or proteomics is preferential. Competitive salary commensurate with training and experience for all positions. Apply online at **website: http://YourFuture.sdbor.edu.** Review of applications will begin September 1, 2006, and continue until positions are filled. *Equal Employment Opportunity/Affirmative Action.*

POSTDOCTORAL POSITION AT EMORY
Angiotensin Converting Enzyme and Tumor Immunity

An NIH-funded Postdoctoral position available to study the role of angiotensin converting enzyme (ACE) in immunity to tumors and viral infections. Mice genetically modified to over-express ACE in macrophages appear very resistant to the growth of melanoma and other tumors. Our observations are very novel and may lead to new insights into immunity and perhaps new immunologically based cancer treatments. At the very least, this project is an excellent vehicle to understand the role of macrophages in tumor immunity. We seek a Ph.D. or M.D. with good familiarity with molecular biology and some familiarity with either immunology or tumor biology. Please contact: **Dr. Ken Bernstein, Room 7107 WMB, 101 Woodruff Circle, Emory University, Atlanta, GA 30322. E-mail: kbernst@emory.edu. Telephone: 404-727-3134.** *Emory is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION
Bacterial Pathogenesis

Postdoctoral position in Streptococcal pathogenesis is available immediately in **Dr. Biswas'** laboratory **(website: http://www.usd.edu/biomed/biomedfaculty/ibw/home.htm).** A doctoral degree with expertise in microbiology, molecular biology, or biochemistry is required. Salary is commensurate with training and experience. Apply online at **website: http://YourFuture.sdbor.edu.** Review of applications will begin September 1, 2006, and continue until position is filled. *Equal Employment Opportunity/Affirmative Action.*

MARKETPLACE